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THE FOURTH REYNOLD A. SPAETH MEMORIAL LECTURE[1]

Genetics and Development

RICHARD GOLDSCHMIDT

Kaiser-Wilhelm-Institut für Biologie, Berlin-Dahlem, Germany

It is one of the sad privileges of men advancing in age to be eligible to the honorable task of delivering lectures dedicated to the memory of a much younger friend whom fate has not permitted to fulfill the great expectations held for him by those who knew him best. In accepting the honor to deliver this memorial lecture, I quite naturally recall the Woods Hole days sixteen years ago, when Reynold A. Spaeth was one of the few with whom I used to discuss certain questions which then were uppermost in my mind. The selection of this evening's topic is therefore influenced by these recollections.

During the summer of 1916 I had the honor to deliver right here in the old lecture hall an evening lecture, in which I tried to explain the experimental results of my work on intersexuality, a term which I had introduced only a year before. I proceeded then to derive from the facts a general theory of sex-determination, which I had developed since 1911, but which had not yet come to be known in this country; a theory which nowadays is called the theory of the genic balance of the sex genes. At the end of this lecture I hinted with a few words at further consequences of the analysis of intersexuality. According to the printed report in the American Naturalist of 1916,[2] I said: "Very important new facts will be published later which will probably enable us to replace the symbolistic Mendelian language, used here, by more definite physico-chemical conceptions." And further: "I am rather optimistic in regard to the general conclusions which might be drawn from these facts, as well as regards the sex-problem as on some fundamental questions of heredity. Combining these facts with the work on hormone ac-

[1] Delivered at the Marine Biological Laboratory, Woods Hole, on August 19, 1932.

[2] Experimental Intersexuality and the Sex-problem. *Am. Nat.*, **50** (1916).

tion as related to sex, we can, I think, form a pretty clear idea about sex differentiation and determination. If we put them in line with the facts of experimental embryology concerning the determination problem we see the outlines of a promising theory of heredity."

During the many years which have since passed, I have tried to formulate the conclusions at which I hinted then and to find new experimental evidence on which to base them. And still, after much thinking on the subject, I stand by the words quoted from 1916, namely: "I am rather optimistic in regard to the general conclusions, etc." The more facts are being accumulated and the more I try to coördinate them and to see a simple guiding idea behind their diversity, the more I am convinced that my method of general approach, which has been highly praised by some and severely criticized by others, is the only one which leads to a deeper insight into the process of heredity. This then is the reason why I have not chosen to present here today some of my recent experimental work, but rather to continue some of the general reasoning from the point where I had left it in my lecture of sixteen years ago.

The decisive step in the analysis of intersexuality, which geneticists often found difficult to understand though physiologists were usually willing to accept it, was that step which led from the static Mendelian analysis of the problem to the dynamic viewpoint of the physiology of development. Here then is found the natural point of departure for our discussion. The limits of ordinary Mendelian analysis, as known at that time, were first reached when it was shown that the experimental facts regarding intersexuality could be expressed not by a simple Mendelian formula, but only by assuming that two genes or completely linked sets of genes,[3]—those for femaleness and maleness,—controlled the result according to their quantitative relation or balance. The simple Mendelian formulation was thus enlarged by a new conception, namely that of a quantitative relation or balance of genes working together towards the production of a phenotype, the character of which was in some way proportional to that quantitative relation of the genes in question—or in other words, their amount of balance or unbalance. This new conception, which had to be added to the general Mendelian formulation and which had given me the clue to the whole analysis already at the beginning of the work between 1911 and 1914,[4] could still be expressed in the old Mendelian language, if the gene in favor of which the

[3] The problem whether only individual sex-determiners or a completely linked group of such are involved in our case, has been repeatedly discussed, *e.g.*, Untersuchungen über Intersexualität V. *Zeitschr. f. indukt. Abst. u. Verer.*, 56 (1930); Analysis of Intersexuality in the Gipsy Moth. *Quart. Rev. Biol.*, 1931.

[4] The whole literature on the subject is found in the author's book: "Die sexuellen Zwischenstufen," J. Springer, Berlin, 1931; further in the paper quoted in footnote 3.

balance acted was called epistatic to the other and if the different degrees of this balance, to which corresponded the sexes and the different types of intersexes, were expressed in terms of degrees of epistasis, which might be measured by some unit. Thus the formulae with numerical values of the genes, symbolizing the grades of their effect, had to be introduced. It took many geneticists a long time to understand this.

But still another extension of Mendelian language was necessary to cover the facts. If the different amounts of the unbalance of male and female genes were to stand for the normal sexes as well as for the different degrees of intersexuality, it followed necessarily that a certain minimum value of this balance had to exist below which one of the pure sexes was determined, and another maximum value, above which the other sex was determined, the intersexual stages lying between. These limiting values for the balance of female and male genes were accordingly called the epistatic minimum, a term which again meant a necessary extension of ordinary Mendelian conceptions, in order to describe the experimental facts still in the language of static Mendelism. This was the point reached in 1912, a point which was situated at the utmost limits of purely Mendelian conceptions. This became clear when the fact was considered that there existed two completely different types of intersexes, namely male and female intersexes, which replaced in the respective experiments the gametic males or females. Now the Mendelian formulation which had covered the case thus far by the introduction of the principle of genic balance and of the epistatic minimum could describe adequately the production of a series of intersexes between the two normal sexes, that is the two limiting minima, but it could not explain why the same ratio between male and female determiners, say the one midway between the ratios for the normal sexes, determined in one case a medium grade female intersex and in another case the completely different medium grade male intersex. Here then was the point at which the power of static Mendelism ended and further progress was only possible by the transition to a dynamic point of view; in other words, the genetic explanation was to be followed by one based upon the physiology of development.

This step, at which I had hinted in the previously mentioned evening lecture given here at Woods Hole, could be taken when it was found what these intersexes really were. It became apparent first in 1916 (and as a matter of fact I do not understand now why I had missed this point in the preceding years) that in a series of intersexes connecting the two pure sexes step by step, such organs which are the last to differentiate in development are the first to assume the character of the opposite sex in the case of low grade intersexuality, and that, vice versa,

the organs which are the first to differentiate in development are the very last to change towards the other sex in high grade intersexuality. From this rule it followed that intersexes are to be considered as individuals which have begun their development as of one sex up to a certain turning-point and have finished it as of the opposite sex after the turning-point;[5] further, that male intersexes begin as males and end as females and that female intersexes begin as females and end as males; and further, that the different grades of intersexuality are a function of the position in time of the turning-point; earlier turning-point—higher grade of intersexuality. This solution which I also had the pleasure to announce first in this country, namely at the 1916 meeting of the American Association, has meanwhile been tested by extensive embryological study and found to be an actual fact. It opened now the way to the solution of the whole problem by connecting a definite embryological process with a definite genetic condition.

The situation was this: On the genetic side we had first a gene or genes for maleness, second a gene or genes for femaleness and both in a series of different conditions, found in different races; further we knew that the phenotypic effect of these genes, namely maleness, femaleness, and all degrees of intersexuality, was proportional to the amount of balance or unbalance of these genes. On the embryological side, we had the occurrence of the turning-point for sexual differentiation at a definite time, and combining now the genetic side with the embryological side, we were facing the fact that a series of increasing values for the unbalance or abnormal ratio of the sex genes has its effect in a series of corresponding changes in the time of incidence of the turning-point, which occurs earlier and earlier. Here then was an opportunity to connect the action of definite genes, present in different ratios, with an embryological event, occurring at definite and proportionally different times. Whereas we have genes for both sexual differentiations simultaneously present, and whereas the control of actual sexual differentiation belonged first to one and later to the other gene or set of genes and whereas this control changes at a definite time, which is conditioned by and proportional to the unbalance or ratio of these genes, there is left only one way of linking these facts together: namely, by concluding first, that the genes in question are producing each independent chains of reaction which lead at a certain threshold to the production of the something which controls sexual differentiation; second, that the amount of unbalance of the two genes or their ratio results in corresponding different velocities of the two chains of reaction; third, that the reaction of

[5] As a matter of fact, Baltzer had found already two years before the same for the intersexes of *Bonellia*, a fact which had escaped me for a long time.

higher velocity controls the sexual differentiation; and fourth, that the turning-points therefore must be the points at which this control changes, which means graphically points of intersection of the two respective curves of reaction. Standing in this place here I cannot help recalling Jacques Loeb's excitement when I told him this story and some of the consequences regarding a general theory of heredity. I have since discussed this point with other great physiologists who agree with me that there is no other way of representing the actual facts from a dynamic point of view. Thus I concluded that here a case was found in which the action of definite genes could and had to be interpreted in terms of speed of reactions and that it might be possible to base a theory of genic action upon this interpretation. Also this conclusion I had the pleasure to announce in this country at the said 1916 meeting of the American Association.

There was also another conclusion which had to be drawn from the same facts, and with this we are getting into deep waters. The phenotypic result (male, female, male intersex, female intersex of any grade) was found to be dependent upon a quantitative relation, balance or ratio of male and female sex genes, and the genetic results showed and have ever since shown that only one female and one male gene are involved. But of each of these sex-genes a considerable number of conditions, in genetic language of multiple allelomorphs, were found which gave typical but different effects. These conditions, which proved to be absolutely constant in all experiments involving the same genes, might be termed the strength of action, or the potency or the valency of these genes, and thus the phenotypic result in regard to sex was dependent upon the relative valencies of the female and male determiners present at fertilization. Then it turned out that one of these determiners, namely the one for maleness, was situated within the X-chromosome; the other one, for femaleness, being outside the X-chromosome. This meant that the always constant genes for femaleness were faced either by one or by two genes for maleness. Thus on one hand, the genotypic effect was produced by the relative valencies of the two types of sex-genes; on the other hand, two of the possible phenotypes, namely the pure sexes, were dependent upon the ratio between the always constant female genes and the male genes present in one or two quantities. In these limiting cases, then, the pure sexes, the relative valencies, responsible for the phenotype, were obviously identical with relative quantities of these genes. But the normal sexes were only two points in a continuous series of sexual conditions, all dependent upon different relative valencies of these genes. The conclusion, therefore, was not only logical but also inevitable that all the other conditions for the sex-

genes, their different degrees in strength or valency, were also in reality differences in their quantity. Thus the quantitative relation or balance of these genes finally was resolved into the ratios of actual quantities. And the former conclusion, which showed that the sex-genes acted through chains of reaction of different but typical velocities, could now be enlarged by the addition that the speed of these chains of reaction is proportional to the quantities of the genes in question.

It is a strange fact that this conclusion was regarded by some orthodox geneticists as a most condemnable heresy. To be sure, they could not give a different explanation of the facts and they could not contest the logic of the analysis. Therefore they simply declared it to be inadmissible—this word has actually been used—to assume that a gene may have a definite and fixed quantity as one of its properties and that the effect of a gene might be in some way proportional to its quantity. In our object, there was no possibility of demonstrating visibly such a difference in quantity, because in our case everything happened within the normal diploid number of chromosomes. But some experiments have since been performed with our material, besides the visible demonstration in triploid intersexuality, discovered by Standfuss and since analyzed in moths and in *Drosophila*. Our experiments in question demonstrate clearly the logic and the soundness of the conclusions.[6] To mention only one: Two X-chromosomes and therefore two male genes (in the case of female heterogamety) are determining the male sex. If, however, I combine female determiners coming from a strong race, that is genes of high valency, with two X-chromosomes derived from races of very low valency of the sex-genes, the resulting individual will be a female in spite of its two X-chromosomes. By appropriate crosses I might now build up individuals which contain the same strong female determiner as before, one X-chromosome with the very low male determiner as before but the other X coming from a race with a little higher grade of valency of the male determiner. The individual thus composed will be a little more male than before, and this is a high grade intersexual male, very near to complete transformation into female. Now I continue replacing the second X-chromosome by one derived from a still stronger and stronger race; correspondingly, the individual in question will be less and less intersexual, so that when a certain combination is reached it will be a normal male. Table I gives an actual experimental result. Now this experiment and its easily imaginable variations show that the action of the two male genes which are present in any case is proportional to the sum of the valencies of the two genes. As a matter of fact, we ought to be able to calculate from a series of such experi-

[6] Details are found in Untersuchungen über Intersexualität, I–V. *Zeitschr. f. indukt. Abst. u. Verer.*, 1920–30; see especially No. V.

ments the relative valencies of all these genes in some arbitrary units, because these experiments furnish a number of equations which may be solved. Thus we have a number of differently active genes and any two of them act together always in proportion to their sum. I can draw from this no other conclusion but that it is the quantity of the thing in question which determines its action.

I have never been able to understand why this conclusion, which safely rests on experimental facts, has been considered by some as offensive. The number, the size, and the shape of the chromosomes are constant; the size of cells is constant and often their number in a given organ; the number and size and arrangement of blastomeres are constant, the number of segments, of bristles, and I know not what else.

TABLE I

Strong F from race	Very weak M from race	Second M from race	♂	Intersexual ♂ grade						♀ (gametic ♂)
				I	II	III	IV	V	VI	
Tokyo	Hokkaido	Hokkaido								
"	"	Berlin								
"	"	Russia								
"	"	Korea								
"	"	Kumamoto								
"	"	Kyoto								
"	"	Tokyo								

Orderly development of a given organism requires a wonderful amount of quantitative constancy from the organ down to the chromosome. Why then should exactly that bit of substance which after all is responsible for all the rest be required to produce its wonderfully typical action of a unique sameness on the basis of a negligible quantity? To my mind, even apart from all the evidence produced, the first requirement for something like an understanding of the action of a thing like a gene would be its presence in typical quantity at the onset, because the mass of a reacting substance is always the first variable to be considered. If in addition, the facts reveal such a simple relation as that between the quantity of the reacting gene substance and a corresponding velocity of reaction, I am ready to consider this as a fundamental insight, upon which one ought to be able to build a theory of the genic action, a theory of heredity.

I have tried now to show how step by step the results of my experiments forced me first to stress the purely Mendelian conceptions by introducing the idea of genic balance and of the epistatic minimum and then to go beyond the limits of static Mendelian conceptions towards the goal of a dynamic understanding of a gene-controlled determinative process. The next step to take was naturally to try to apply the fundamental conceptions to the elaboration of a general theory of heredity, based on the principle of coördinated reaction velocities, as announced in my lecture here sixteen years ago.

It is only recently that I learned[7] that a few years before I had derived my conclusions and had embarked upon their generalization, Professor M. F. Guyer had already arrived at a similar conception which, though no experiments were available at that time, was developed by him in a very ingenious way. I am glad to make use of this occasion to pay my respects to Professor Guyer's intuition and to quote some of his sentences, namely:

"If in the comparatively simple cases of associated simultaneous reactions with which we are acquainted in non-living matter, relative velocities may so modify the results, we can readily realize of what tremendous importance regulation of this matter must become in living protoplasm where doubtless vast numbers of chemical reactions and interactions are going on at the same time. In fact, could we locate such a time-regulating factor in the germ-cell it would seem that we had accomplished a long stride toward an understanding of the controlling and coördinating mechanism which insures the appearance of just the proper substance at the right time in morphogenesis. It would constitute a qualitative as well as a quantitative regulator, for by determining quantity at any given time it determines what the next chemical reaction will be, and hence in the very doing of this, it necessarily conditions the chemical outcome of that reaction."

There can be no doubt that these sentences contain already the essence of the theory of the orderly arranged, interwoven, and balanced velocities of reaction. Returning now to the further development of my own work, I obviously continued arguing the following way: Determinative processes in regard to sex have to do with almost any type of morphological and physiological differentiation occurring in development. If, for example, we turn our attention to a single organ like the genital armature in insects, which exhibits differences in the two sexes, of a degree, which might be compared to the differences in structure of two far distant organisms, we realise the amount and diversity of specific differentiation which may be brought about by such a simple system of coördinated reaction velocities as that which had been actually demonstrated. And if we include in this deliberation all the complex forms of one and the same organ which are obtained in a thoroughly orderly fashion in case of intersexuality, which means in consequence of a change in the coördination of the system of reaction velocities, we come to the conclusion that a similar conception ought to be applied to all

[7] Guyer, M. F. The Germinal Background of Somatic Modifications. *Science*, 71 (1930).

types of morphogenetic processes, that is, to development in general. Development ought to be disentangled into a series of coördinated reactions of definite velocities, producing at a certain threshold a certain event, say the appearance of embryonic hormones or of determining stuffs, thus securing the order and seriation of developmental processes. And just as in the intersexuality experiments the genes in question controlled the respective speeds of reaction, so in normal development would the genes also control the speed of reactions with which they are concerned. Expressed more specifically, the genes must be things which produce their typical effects by catalyzing chains of reaction, the speed of which, ceteris paribus, and given the specific substance of each gene and the plasmatic substratum, is proportional to the quantity of the gene and therefore fixed within the entire system of simultaneous coördinated reactions of different speed.[8]

We have tried since to demonstrate in detail how such a system accounts not only for numerous genetic facts, but also for facts of experimental embryology; and indeed even sheds light on evolutionary questions. I shall not try now to develop these conclusions, as it is my intention this evening to discuss in the first line the experimental and logical basis of the whole argument. The principle will, moreover, be visible incidentally if I continue relating the actual sequence of findings which helped to shape these ideas. The different sex-genes of typical valency or quantity behaved in the experiments as a series of multiple allelomorphs, of which 8–10 members have been isolated by now.[9] Simultaneously I was studying another series of multiple allelomorphs which permitted the analysis of the effects of the genes within this series in a dynamic way, because the effect of these genes became visible in the larvae of *Lymantria*.[10] There were found races in which young caterpillars were dark and remained so through all instars. There were others which had light markings and which remained light through all instars. And there were again others which were light in the young stages and turned dark in later instars. Between these extremes all transitions were found as the curves of pigmentation show, and each of these types is produced by a member of a series of multiple allelomorphs.

[8] These views and their consequences have been developed in: Die quantitative Grundlage von Vererbung und Artbildung. *Roux's Vorträge u. Aufsätze Entw. mech.*, **24** (1920). A more detailed account, leaving out the evolutionary side, is found in: "Physiologische Theorie der Vererbung," Springer, Berlin, 1927.

[9] Final data in: Untersuchungen zur Genetik der geographischen Variation III. *Roux's Arch. Entw. mech.*, 1932.

[10] Short accounts of the main facts were published in: A Preliminary Report on some Experiments concerning Evolution. *Am. Nat.*, **52** (1917); and: Die quantitative Grundlage etc. (see footnote 8). A detailed report is found in: Untersuchungen zur Genetik der geographischen Variation. I. *Roux' Arch. Entw. mech.*, **101** (1924); Also, II, Ibid., **116** (1929). Consult this for photos and curves.

A closer study of the facts then reveals that each allelomorph of the series is responsible for a process of accumulation of dark pigment on the basis of light markings, a process which proceeds with a definite velocity which is typical but different for different allelomorphs of the series, as may be demonstrated in a diagrammatic curve.[10] Here then we found again a series of multiple allelomorphs connected with a series of reactions of different velocities, and we concluded that also this series, and, perhaps, most similar series, must consist in one and the same gene in different quantities. In this case, of course, the conclusion rests on analogy, and no way to prove it is apparent. This case, however, furnished another fact which pointed in the direction of the general theory. If we cross the always light race with the always dark race, the young F_1 caterpillars are first light, but later they become dark. In Mendelian language, light was first dominant and later dark. If we remember the last curves, it is clear that the curve which is midway between the ones of the light and dark races has exactly this type, first light, later dark; and as a matter of fact the intermediate allelomorphs of the series also produce the same effect as observed here in the hybrid. This then shows clearly that dominance, recessivity, and change of dominance are here the phenotypic effects of the type of reaction curve within the whole system. From this fact then may be derived a few theoretical cases which simultaneously are apt to serve as a model for the whole generalization.

Let us consider what dominance might mean within a system of genes which are responsible for reactions with velocities in proportion to the quantity of the genes. Figure 1 [11] assumes that we consider two allelomorphs, each producing a reaction of different velocity represented by straight lines. At a certain level or threshold marked by the line M, the determinative reaction takes place. Let us now assume that we are dealing with the size of an organ which is the result of a given number of successive cell-divisions. The reaction in question may stop the cell divisions and therefore the resulting size of the organ will be smaller and smaller, the more early the reaction curve reaches the level M. If the cell divisions proceed with equal time intervals and if the reaction velocity for the heterozygote is per definitionem intermediate between the two parents, the size of the organ will also be intermediate. Let us now assume that the cell divisions in question proceed first slower and then faster, as represented on the line M_1; the same system leads then to almost complete dominance of the greater size; if, however, cell divisions proceed first faster and then slower as represented on line M_2, we find almost complete dominance of the smaller size.

[11] Taken from "Physiologische Theorie der Vererbung," 1927.

I think that this diagram which follows immediately from the preceding analysis, is rather instructive. It demonstrates a simple interpretation of dominance; furthermore, we have to assume that the three forementioned types of cell division are themselves determined directly or indirectly by the action of other genes, which in genetic language are usually called modifiers. Dominance then is the result of the interaction in time of the heterozygous main gene with a number of others, the modifiers. Those among you who are acquainted with Fisher's so-called theory of the origin of dominance will realize at once that only such a system, as presented here, will allow that dominance is changed

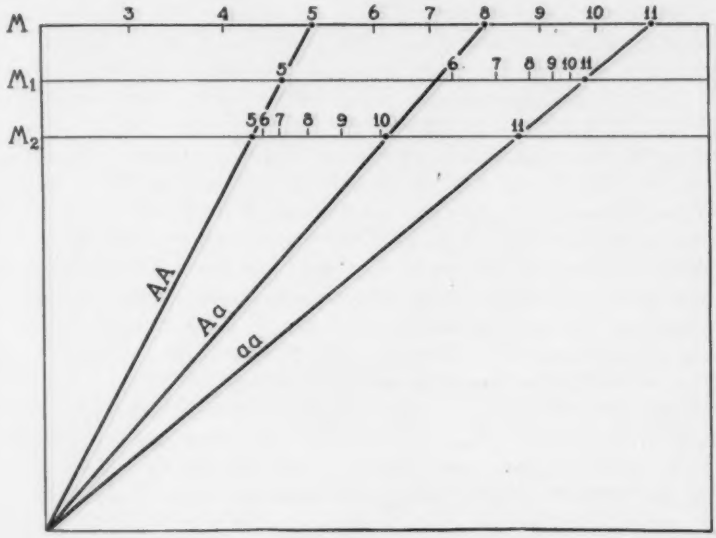


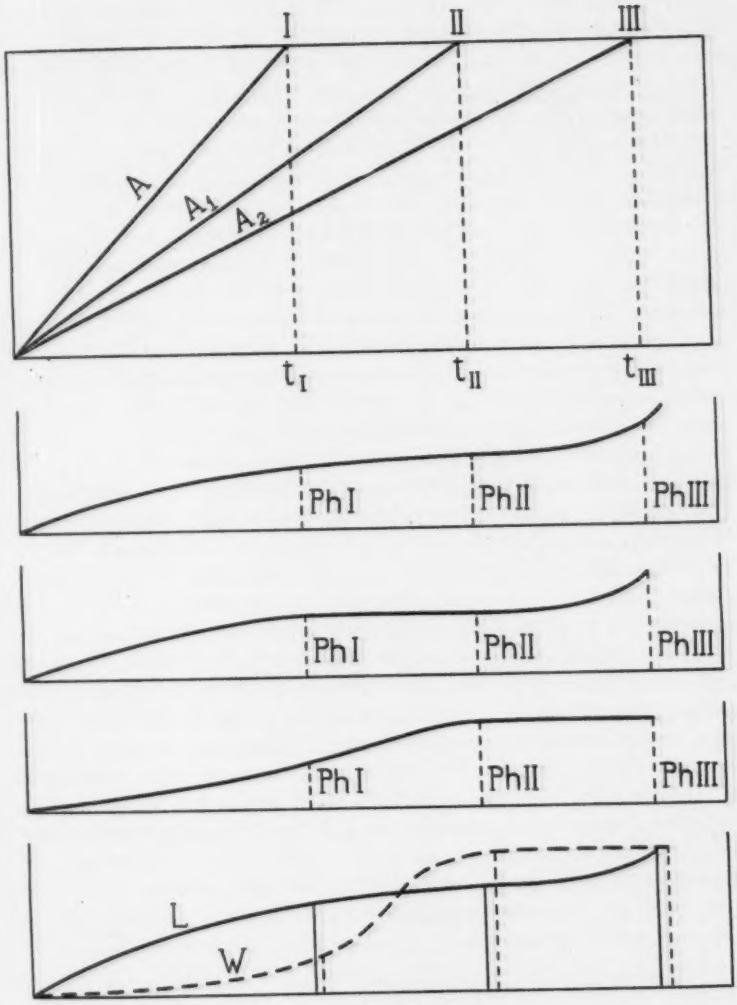
FIG. 1

by selection of modifiers. Moreover, the diagram may be used as a model for all possible determinations of developmental processes into which the embryology of an individual might be dissolved. By changing the meaning of the variables, introducing new ones, or other threshold conditions, similar models might be derived for all kinds of facts relating differentiation to genic action. Finally, the diagram may show that it is of no use to discuss the problem of the quantity of the gene without considering the corresponding reaction velocities through which alone the assumption of different but typical gene-quantities becomes important; because without this connection we have only a sterile hypothesis. Let

me illustrate finally this point by an actual case. Dobzhansky [12] some time ago set out to disprove the quantitative nature of multiple allelomorphs in the following way: He argued that if we consider a series of multiple allelomorphs which produces manifold phenotypic effects in different organs, these effects must show always a parallel seriation in different combinations of these allelomorphs, if the genes in question form a quantitative series. A study of the facts did not prove this to be the case, and therefore he concluded that the allelomorphs cannot be of a simple quantitative nature. As a matter of fact the premises of this argument are already wrong, because the main point has been neglected: namely, the system of reaction velocities. This will be evident at once if we consult again a similar diagram as before (Fig. 2). We have represented three allelomorphs by their reactions of different velocities which lead to a determinative effect at a certain threshold after the times I, II, III. Let us assume again a very simple type of effect, namely the cessation of growth of an organ at the time in question. Each organ of which the size is influenced by the series of allelomorphs may, of course, have its own curve of differentiation which is determined independently of the allelomorphs in question. In order not to complicate the diagram, some of such possible curves have been drawn below. The size of the organ reached at the decisive times I, II, III is then represented by the verticals Ph I, II, III. In the first case, the organ shows a steady increase with the three allelomorphs in question; in the second organ the first two allelomorphs produce the same effect; in the third organ the effect is identical for the second and third allelomorph. The fourth case represents the growth of an organ in two dimensions represented by a length-breadth index. *L* is the curve for a constant growth in length, *W* the curve for intermittent growth in breadth, and the proportion of the two verticals at time I, II, III the respective index of the resulting phenotype. In the case which is represented, this index is first high, then low, and then again higher. This simple diagram shows then how in such a system of timed reactions a series of causes of a definite order, for example a set of different quantities of a gene, might produce effects of a very different order in different organs.

A third example of the application of the general idea might be discussed which is to be regarded as representative for a certain group of problems. The wing of butterflies and moths constitutes after a certain critical period, which is situated towards the end of the larval stage, a self-differentiating system. A nice demonstration of this I was able to

[12] Dobzhansky, Th. The Manifold Effects of the Genes Stubble and Stubbloid in Drosophila melanogaster. *Zeitschr. f. indukt. Abst. u. Verer.*, **54** (1930). Goldschmidt, R. "Bemerkungen zur Kritik der quantitativen Natur multipler Allele." Philiptschenko Gedächtnisband, Leningrad, 1932.


I
II
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L
W

FIG. 2

give many years ago [13] when I showed that it is possible to change the speed of differentiation of one wing without altering the other wing of the same individual at all, namely by blocking the blood supply to a certain extent. We may have side by side the normal wing which has almost finished its differentiation and the operated wing of the same animal which shows structure and coloration of an earlier larval period. (These experiments, by the way, have anticipated the general type of some recent experiments performed on amphibian eggs with local temperature changes.) This self-differentiating system of the wing pattern is finally determined during the critical period. At this time, when the wing is a simple epithelial sac showing no visible differentiation on its surface which would correspond to a later pattern, the future pattern is already completely laid out. How this is done we do not yet know. But two significant facts have come to light which may be regarded as the beginning of an understanding. One relates to the wing of intersexual males of the gipsy-moth. Such a wing exhibits the characteristic mosaic streaks of white female colour upon the brown male wing. If these white patches are large, it can be shown that they have also a different rate of growth from the brown areas. At the time of pupation, of course, no such structure can be seen on the epithelial wing, but in some cases the wing-mosaic may be faintly but clearly seen on the pupa-case, which has been secreted by the wing epithelium. This shows that the pattern is already present in the form of some difference in regard to the secreting activity or some other process involved in the formation of chitin by the wing-surface. A little later, however, but a long time before any pigment appears, the difference in question can be made visible, and it may be shown in what it really consists. That is, the prospective white parts of the wing are far in advance of the later dark parts in regard to the differentiation of the scales. This may be made visible by drying the wing which has been taken out of the pupa. The prospective white parts carry well-chitinized scales which remain erect when drying; the future dark parts, however, are still carrying younger soft scales which collapse in drying, so that on a wing treated in such a way the future white parts stand out in relief.[14] This then shows in one case that the primary pattern formation consists in producing areas with different speed of differentiation. These findings in the intersexual wing

[13] Untersuchungen zur Entwicklungsphysiologie des Flügelmusters der Schmetterlinge. *Arch. Entw. mech.*, **47** (1920).

[14] For particulars and discussions see: Untersuchungen über Intersexualität. II. *Zeitschr. f. indukt. Abst. u. Verer.*, **29** (1922). Einige Materialien zur Theorie der abgestimmten Reaktionsgeschwindigkeiten. *Arch. Entw. mech.*, **98** (1923). The majority of the extensive studies of the author and his former student F. Süffert have never been published. The same principle has been always found at work.

proved further to be in full harmony with other results in regard to the development of the wing pattern, which had been found in other objects. I could show that the normal wing pattern in many different types of butterflies and moths is laid down in the same way,[15] namely, as regions of different speed of differentiation.

The following slide showed a swallow-tail *Thais polyxena* with its characteristic pattern and besides a wing taken from a pupa, before any pigment becomes visible. In drying the wing, the ghost-pattern becomes visible because the future light scales remain erect, but the future pigmented scales collapse. The photograph does not allow it to be distinguished clearly that no pigment at all is involved in this picture. This is more easily visible in another picture representing an unpigmented pupa wing of a Cecropia moth. The white margin of the eye spot is easily seen as a group of erect scales, whereas those of the dark spot are collapsed.

The second important fact has recently been found by a student of Professor Kühn.[16] In the larval wings of the meal-moth at about the critical period he found zones of intense mitotic divisions, which corresponded to later elements of the wing pattern, elements which later follow the same law which we just described. Though it is not yet possible to coördinate and to understand all these facts, they might be represented in general terms at present in the following way: In the critical period which corresponds to the time of irreversible determination found in each study of developmental physiology of any organ, a pattern appears of physiologically different areas on the wing, different in regard to their growing activities and to their relative speeds of differentiation. This suggests the appearance and typical distribution of something like a growth hormone. All the rest of the differentiation of the pattern, however, is nothing but the consequence of a coördinated system of reaction velocities in regard to differentiation and also to chemism. Figure 3 may serve as a model for the whole process, which might be varied indefinitely to fit individual cases. We assume that the wing area differentiates during the critical period into three different parts, according to what we have seen before. Each of these areas, I, II, III, begins to differentiate at a different rate represented by the three curves T_1, T_2, T_3. At the level of the points T_1, T_2, T_3 the respective scales have reached the stage or threshold which permits of the deposition of pigments. We then see three independent gene-controlled chains of reaction which are supposed to result in the formation of some component, requisite for the final deposition of yellow, red, and black pigment respectively within the scales at the times Tp_1, Tp_2, Tp_3. Now at the time

[15] Papers quoted in footnotes 13 and 14, see further: "Physiologische Theorie der Vererbung."

[16] Köhler, W. Die Entwicklung der Flügel bei der Mehlmotte Ephestia Kühniella Zeller, mit besonderer Berücksichtigung des Zeichnungsmusters. *Zeitschr. f. Morph. u. Ökol. Tiere*, **24** (1932).

Tp_1 only the area I is ready to receive the stuff P_1, and therefore only this area will contain yellow scales; similarly for the two other areas. It is clear that this diagram, which is based on the actual facts, may be varied to fit any type of pattern, pigment etc., and that it might be as well used as a model for many processes of determination which after all are nothing but formations of patterns.

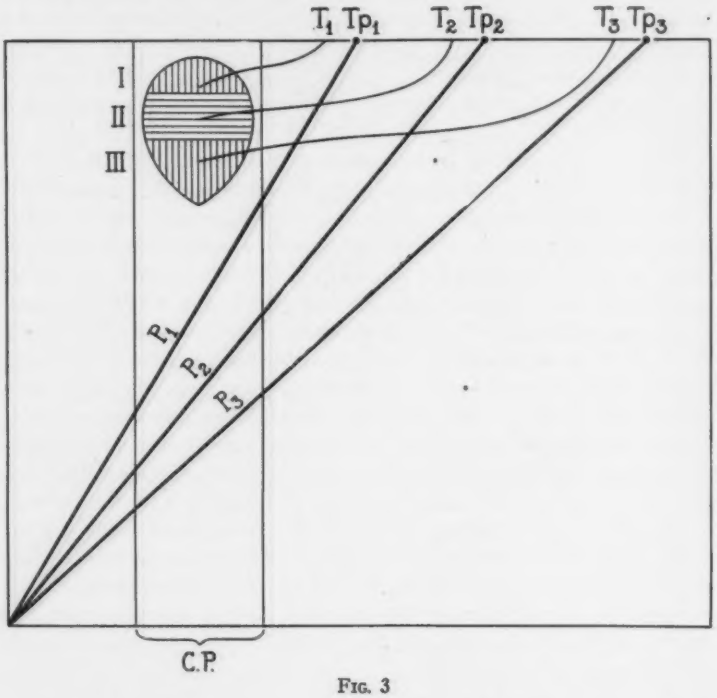


FIG. 3

Only one of the consequences may be mentioned, partly because it is connected with some of our own work, partly because it opens up vistas in another direction. The classic temperature experiments with butterflies have shown that it is possible to change the inherited wing pattern by applying extreme temperatures and other extreme conditions to the animal within the critical period, the duration of which has been exactly determined.[17] One of the well-known results of this old work, which

[17] The well-known work of Standfuss, Weismann, Fischer, Merrifield. Determination of the critical period by my former student F. Süffert: Bestimmungsfaktoren des Zeichnungsmusters beim Saison-Dimorphismus von Araschnia levana prorsa. *Biol. Centrbl.*, **44** (1924)

we have repeated on a large scale, is the fact that in a number of cases it was possible to produce in the temperature experiments forms as non-heritable modifications, which are phenotypically identical with well-known geographic subspecies, a fact which plays a considerable rôle in Lamarckian discussions. A typical case is that of *Vanessa urticae* from the European continent and the subspecies *V. ichnusa* from Mediterranean islands; the phenotype of the latter is exactly reproduced in the temperature experiments with the former. Many similar cases are known. Figure 4 gives the type of explanation of such cases,[18] I re-

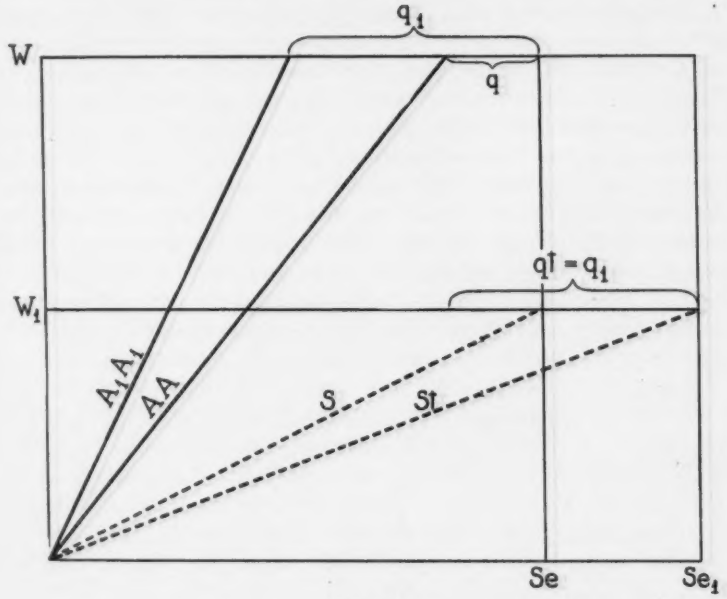


FIG. 4

peat, the type, because no actual analysis has been made which would show which individual reactions are concerned with the special case. The diagram, therefore, does not claim to cover the actual case but to represent the type of explanation which has to be applied, all details being indefinitely variable to fit the individual case. We assume that the phenotypic differences of the two forms in question are differences in the area which one definite element of the pattern occupies. This rela-

[18] Untersuchungen zur Entwicklungsphysiologie des Flügelmusters der Schmetterlinge. *Arch. Entw. mech.*, **47** (1920).

tive area is determined during the critical period which is supposed to end at the time *Se*. One of the simplest possibilities for the determination of the size of this area is, that it is proportional to the time which is available from the beginning of its formation to its final determination with the end of the critical period. Both of these points are, of course, determined independently and genetically, and we express this by assuming a genetic chain of reactions *AA* which reaches its active minimum at the level *W*, and a second chain *S* which determines similarly the time at which the critical period ends, *Se*. The distance between the two times, *q*, then is proportional to the area of the pattern-element in question. Now we might have another race in which genetically the curve *AA* is replaced by A_1 A_1, and therefore the area of the pattern in question is proportional to the distance q_1, that is, bigger. If I perform now a temperature experiment during the critical period, and the *S* and *A* chains have a different temperature-coefficient, I might shift *Se* to Se_1, without touching *AA*. Now our area is proportional to the distance *qt* which is equal to q_1, and the phenotype is exactly identical with the one of the race A_1. Speaking generally, we learn from this diagram that it may be possible within a system of timed reactions to produce a certain new phenotype by shifting one of the reactions, by changing its velocity. This shifting, however, and therefore the same effect, may be due either to an external agency like temperature, or to a mutation of the gene which lies at the basis of the reaction in question.

There is one consequence of these considerations which seems rather important. In such a system of timed reactions, there are not many degrees of freedom imaginable for the individual reaction, which would not upset the whole system. Therefore viable mutations are limited, and furthermore within such a system viable mutations are only imaginable, the phenotype of which might theoretically also be obtained by proper external action as modifications. This means that if we know the proper agents and the proper critical periods, we ought to be able to produce also the phenotype of every imaginable or known mutation in the form of a non-heritable modification. Putting aside the manifold obvious cases of this type in quantitative characters like size, I might mention that I succeeded in producing the exact phenotype of a considerable number of *Drosophila*-mutations as non-inheritable modifications through the action of extreme temperatures at different critical periods. It is very significant that in such experiments usually the modification in question appears simultaneously in a series of degrees, paralleling exactly series of known or also not yet known multiple

allelomorphs.[19] If we remember what we heard before about such series and the reaction velocities, the wonderful consistency of all the facts and their connection through a rather simple idea becomes once more apparent.

I do not think that much imagination is needed to apply the different models of the argument, which have now been discussed, to any imaginable process of differentiation which proceeds orderly with time, and I believe that the relation between the gene and that part of the process of embryonic differentiation which belongs to the dimension of time is adequately explained by the system of timed reactions and what belongs to it. This, however, is only a part of the problem of embryonic determination. There is, in addition, the differentiation of the substratum in the three dimensions of space without which the reaction system which produces the right thing at the right time could not be imagined to produce it also in the right place. There can be no doubt that the spatial differentiation of the substratum is also produced at definite times by the same system of genic and timed reactions. Under normal conditions, a certain embryonic area, say a limb-bud, is equipotential up to one moment and differentiated into parts of different potency from that moment on. And this time of determination may be different but genetically fixed in nearly related species. We have discussed this point already in regard to the wing pattern. Further, all the elementary facts of experimental embryology, beginning with the analysis of the different types of eggs in regard to determination, prove that the progress of differentiation may be dissolved into a series of exactly timed events, consisting mainly in some diversification of the substratum, be this the egg which is to be regarded as an individual system, be it progressively smaller and smaller areas of the embryo, now to be regarded as the individual systems, which change at a certain moment from a monophasic to a polyphasic condition.[20] The causation of this change still belongs to the domain of physiological genetics, and is adequately understood by the system of timed reaction velocities. But in what this change consists and what are its consequences in regard to determination,—this is the proper domain of experimental embryology. The experimental facts have been described under many headings since the days when His first understood the problem with a really prophetic vision. Organ-forming stuffs, chemo-differentiation, embryonic segregation, and the organizator are all terms for the observed facts of the same order. The organizator

[19] Only a short notice has been published, though a considerable material has been accumulated. Jollos, who has repeated the experiments with the same results, is preparing a communication which relieves me from publishing the details of my results.

[20] Detailed discussion in "Physiologische Theorie der Vererbung."

conception, in addition, has led an important step further, because it connects the facts of the diversification of the substratum with former causative events and therefore opens the way for a dynamic understanding of a sequence of events, which has been started at one point. And the theory of the metabolic gradients, which constitutes the physiological corollary to the morphological organizator-concept, makes visible one of the ways for a causal explanation of the whole process.

Whatever this process of the diversification of the substratum, or in one word including all visible types, the process of stratification, might be, its meaning within the genetic system of timed reactions is clear. It allows the products of the genic reactions to act or not to act or to act differently on different areas of the germ, it creates secondary and tertiary systems, influencing the course of the genic reactions differently in the different regions, allowing one and the same original chain of reactions to lead to different consequences in the different areas, and the same over and over again up to the end of differentiation.

It would be pleasant to point to a few of the consequences which might be derived from such views as the ones presented here, consequences in regard to special and general problems of genetics, problems of mutation, evolution, the understanding of rudimentary organs or embryonic recapitulation. But these conclusions may be easily drawn by anybody who is willing to accept the soundness of the basic idea.[21]

Ladies and Gentlemen! A few years ago, one of the leading biologists of this country professed right here his opinion that the time has not yet come for genetics to join hands with experimental embryology. Permit me to conclude this lecture by expressing most emphatically my conviction that not only this time has long since come, but also that the foundations for an understanding of development from the standpoint of physiological genetics have already been laid. Indeed a considerable part of the frame-work stands ready around which to erect a good building.

[21] Some of them have been presented in Die quantitative Grundlage etc., Materialien zur Theorie etc., and " Physiologische Theorie, etc." quoted before; others have meanwhile been drawn by other authors, who accepted the general trend of our ideas.

THE ORIGIN AND BEHAVIOUR OF CHIASMATA

V. *Chorthippus elegans*

C. D. DARLINGTON

John Innes Horticultural Institution, Merton, London

Introduction

At the pachytene stage of the prophase of meiosis the chromosomes are single threads and these threads lie side by side in pairs throughout their length: association is complete. But at the end of this stage the threads fall apart; they appear to repel one another and they are found to be longitudinally double, owing to the division of each chromosome into two "chromatids." The double threads remain associated, however, in spite of their repulsion, for the chromatids change partners at one or more places which are known as "chiasmata." The chiasmata persist with various changes in number and position from this diplotene stage until metaphase and they are important, first, on account of their function in holding the chromosomes together and allowing them to continue paired until metaphase, and, secondly, on account of their being the result of genetic crossing over between chromatids of opposite chromosomes (*cf.* Darlington, 1932). In order to understand their cause and function properly it is necessary to record their varying numbers and positions at successive stages of prophase in large numbers of organisms and so deduce their behaviour and the forces determining their origin and maintenance.

These forces have very different effects in different organisms and the subject of the present study, *Chorthippus elegans* (Acrididæ) is an example of a species with the least possible change in the number and position of the chiasmata between diplotene and metaphase. The methods used were the same as in *Stenobothrus* (Darlington and Dark, 1932) except that all the observations are from one smear preparation of one individual (giving the maximum uniformity of material). This was stained with Heidenhain's hæmatoxylin instead of gentian violet; the result, though less satisfactory for tracing the course of the separate chromatids at diplotene, is relatively free from danger of fading.

Observations of Spermatogenesis

Pachytene.—Eight double threads, the paired autosomes, can be distinguished together with one more condensed and homogeneous body,

the X chromosome. No doubleness can be definitely made out in the autosome threads until the end of this stage, but the upturned ends of chromosomes show what may be the beginnings of a split. The X

FIGS. 1–12. The eight paired autosomes and the X chromosome at successive stages of prophase. The total number of chiasmata and the number terminal are given under each bivalent in the later stages. × ca. 2000.

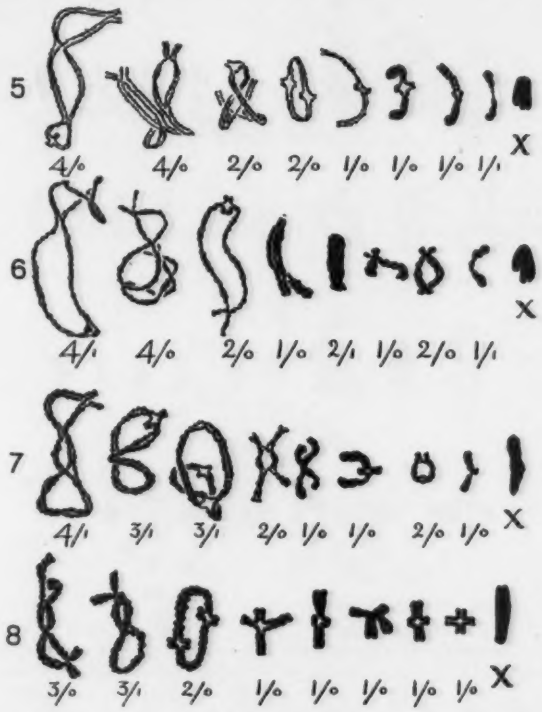
FIG. 1. The end of pachytene.

FIG. 2. The beginning of diplotene. Separation of the shorter chromosomes complete.

FIGS. 3 and 4. All loops completely open and chiasmata recognizable.

chromosome, however, has already divided into two halves. There is little evidence of a "bouquet" stage at zygotene and none persists through pachytene.

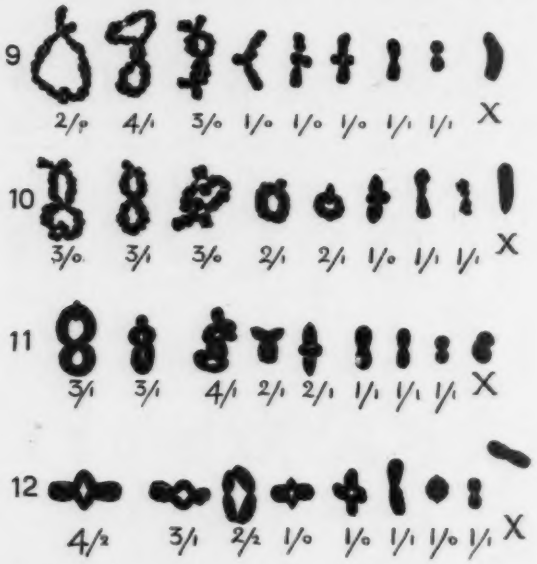
Diplotene to Diakinesis.—The paired threads fall apart (Fig. 1). The long chromosomes with median constrictions begin to separate either in the middle or at one end. Doubleness is still not evident.[1] The separation is first complete in the short chromosomes (Fig. 2). These are also found to be slightly in advance of the longer ones in condensation (Fig. 3) and they maintain this difference until diakinesis. This suggests that differential precocity ("heteropycnosis") here de-



FIGS. 5–8. Successive stages of diplotene.

[1] Robertson and others have stated that the chromosomes are double before they pair but do not claim to have any direct evidence to show that this is so. The *indirect* evidence they bring forward consists in the well-known supposition of "anaphase duality" which is now seen to depend on a misinterpretation of spiral structure (Darlington, 1932). On the other hand, all *direct* observers of zygotene in plants and animals agree that the chromosomes are single at this stage (Gelei, Wenrich, Belling, Newton and Darlington). Speculations as to the possible earlier division of the chromosomes at meiosis and mitosis conflict with the precocity theory of meiosis (Darlington, 1931) but are not valid evidence against it.

pends on size differences. The loops first meet, revealing the number and position of the chiasmata, in the shorter pairs. As the loops open the doubleness of the chromosomes becomes detectable (Fig. 2). The chromatids can then be followed separately through the chiasmata and sometimes throughout their length. Later they swell considerably and cease to be separately identifiable, except at the chiasmata (Fig. 9). This swelling is seen in many animals (perhaps *Pristiurus* may be regarded as the extreme type) and also in a gymnosperm, *Taxus baccata*



FIGS. 9 and 10. Diakinesis.
FIG. 11. Pro-metaphase.
FIG. 12. Metaphase, X chromosome lying to one side of the spindle. *Note:* the three long pairs of chromosomes have median spindle attachments, the rest terminal.

(Dark, unpublished). It is characteristically different from the behaviour in the angiosperms where the more swollen condition prevails throughout the post-pachytene stages and seems to prevent the chromatids being so clearly distinguished at diplotene as in some Orthoptera.

The differences between angiosperm and orthopteran may, of course, be artefacts but it is impossible to say which is normal and which the artefact. Many would infer that the clearer observation is more true-to-life but such a conclusion does not necessarily follow. The increase in size has a curious effect. Owing to the differential but somewhat

variable rate of condensation of short and long chromosomes, their relative sizes during the diplotene phase change rather suddenly and interfere with the constant distinction of types (Figs. 3–6).

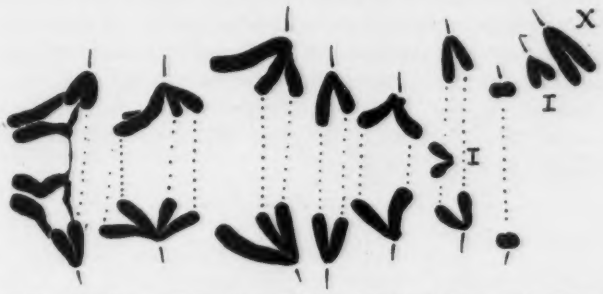


FIG. 13. Anaphase of the first division following failure of pairing of the shortest medium pair; one of the univalents is about to divide at the first division. The chromatids that have been associated are connected by a dotted line. × ca. 3000.

FIGS. 14 and 15. Second division. × ca. 3000.

FIG. 14. Polar view of the eight autosomes in metaphase.

FIG. 15. The two divisions of one spermatocyte. Above, anaphase; nine chromosomes including the X chromosome. Below, metaphase (chromosomes drawn separately); the distal ends of some of the chromosomes are coming together.

Metaphase to Anaphase.—Between pachytene and metaphase the chromosomes contract to about one-sixth their length. The eight bivalents lie on the equatorial plate and the X chromosomes to one side

of it (Fig. 12). Repelling one another from their spindle attachments the pairs of chromatids associated at these points pass to opposite poles. Those pairs distal to the first chiasma therefore have to separate and the strain often draws them into a fine thread. Exceptionally a connection is seen between the separating chromatids (Fig. 13), as already noticed in *Hyacinthus* and *Stenobothrus*. This connection is as yet unexplained. Exceptionally also two of the shorter chromosomes are found to be unpaired and may lag and divide at the first division. This failure corresponds with observations during prophase and, as will be seen, is related to conditions of chiasma formation.

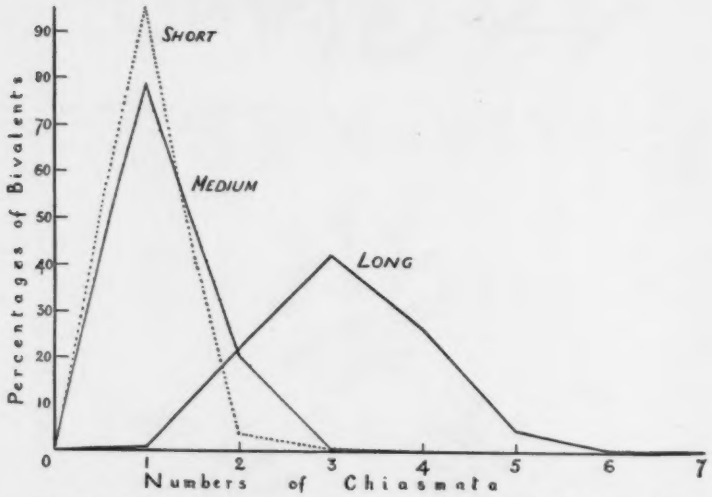


FIG. 16. Graph showing variation in chiasma frequency in the long, medium, and short types of chromosome.

Second Division.—During the interphase and early metaphase of the second division the pairs of chromatids are held together only at their spindle attachments (Fig. 14). They suddenly come together, immediately before anaphase, it must be presumed, for they are rarely seen together during metaphase and then only touching at the ends (Fig. 15).

Syndiploidy.—Groups of from two to six spermatocyte nuclei are often found closely appressed, between pachytene and diakinesis; and at metaphase, in a corresponding proportion of cells, fusion of the adjoining plates of chromosomes is seen. Where only two nuclei lie together the result is a regular fused spindle but, with a larger number, the chromosomes no longer orientate themselves regularly. These re-

sults are closely paralleled by Eisentraut's observations (1926) on *Gomphocerus maculatus* and by numerous observations of syndiploidy in plants, although the exact time of fusion, the onset of metaphase, is not elsewhere so clear. It is evident that in all these cases fusion takes place after pachytene and, although four homologous chromosomes of each type are present (in both plants and animals), quadrivalents are

TABLE I

Summary of Observations of 100 Nuclei (800 Bivalents)

Numbers of Terminal Chiasmata in each Bivalent		Numbers of Chiasmata per Bivalent											
		long type (three)						medium type (four)				short type (one)	
		1	2	3	4	5	6	0	1	2	3	1	2
Mid Diplotene (24 nuclei)	0	—	11	20	9	4	—	—	67	11	—	8	—
	1	1	3	9	7	2	—	—	3	15	—	14	2
	2	—	1	2	1	1	1	—	—	—	—	—	—
Total		1	15	31	17	7	1	—	70	26	—	22	2
Late Diplotene (16 nuclei)	0	—	8	10	4	—	—	—	41	7	—	3	—
	1	—	3	13	7	—	—	—	10	6	—	13	—
	2	—	—	1	—	2	—	—	—	—	—	—	—
Total		—	11	24	11	2	—	—	51	13	—	16	—
Diakinesis (40 nuclei)	0	2	14	17	2	1	—	1	90	—	—	4	—
	1	—	12	30	15	2	—	—	43	25	1	35	1
	2	—	1	12	10	2	—	—	—	—	—	—	—
Total		2	27	59	27	5	—	1	133	25	1	39	1
Metaphase (20 nuclei)	0	—	3	3	1	—	—	—	31	—	—	4	—
	1	—	9	12	9	—	—	—	30	18	1	15	1
	2	—	1	8	14	—	—	—	—	—	—	—	—
Total		—	13	23	24	—	—	—	61	18	1	19	1
Grand Total.....		3	66	137	79	14	1	1	315	82	2	96	4
Percentages		1	22	45.7	26.3	4.7	0.3	0.3	78.7	20.5	0.5	96	4

never formed. Evidently, therefore, the pairing of chromosomes at metaphase in these organisms can only be derived from pachytene pairing and does not arise from a direct affinity. This conclusion follows from the chiasma theory of pairing and contradicts the assumption that the chromosomes are paired at metaphase on account of any attraction operating between them at this stage (Darlington, 1931).

Chiasma Frequency.—The numbers of chiasmata present in the bivalents of each type remain without significant change from diplotene to metaphase. They show (Fig. 16) the interference curves of frequency variation constantly observed in all organisms so far studied (Haldane, 1931; *cf.* Darlington, 1932). They also show (Table II) the indirect relationship of length of chromosome to frequency already found in *Stenobothrus* and probably very general in organisms with

TABLE II

Mean Chiasma Frequencies per Bivalent (derived from Table I)

		Mid Diplotene Stage (24 nuclei)	Late Diplotene Stage (16 nuclei)	Diakinesis (40 nuclei)	Metaphase (20 nuclei)	Total (100 nuclei)	Observations on Stenobothrus parallelus	Length
Long Type	No. Bivalents...	72	48	120	60	300		11.3 μ
	Total No. Chiasmata.....	233	148	366	191	938		
	No. Terminal Chiasmata....	34	29	109	76	—		
	Chiasma Frequency per Bivalent.....	3.24	3.08	3.05	3.18	3.13	3.31	
	Terminal Chiasmata per Bivalent.....	.47	.60	.91	1.27	—		
Medium Type	No. Bivalents...	96	64	160	80	400		4.1 μ
	Total No. Chiasmata.....	122	77	186	100	485		
	No. Terminal Chiasmata....	18	16	69	49	—		
	Chiasma Frequency per Bivalent.....	1.27	1.20	1.16	1.25	1.21	1.45	
	Terminal Chiasmata per Bivalent.....	.18	.25	.43	.61	—		
Short Type	No. Bivalents...	24	16	40	20	100		1.6 μ
	Total No. Chiasmata.....	26	16	41	21	104		
	No. Terminal Chiasmata....	16	13	36	16	—		
	Chiasma Frequency per Bivalent.....	1.08	1.00	1.02	1.05	1.04	1.04	
	Terminal Chiasmata per Bivalent.....	.61	.81	.90	.80	—		

wide range of size amongst the chromosomes (Darlington and Dark, 1932). This effect is not so pronounced, however, in the medium chromosomes as in *Stenobothrus* with the predictable result (on the chiasma theory of pairing) that the shortest member of this type occasionally fails to pair (Fig. 13, anaphase, and Table I, diakinesis).

Terminalization.—The total number of chiasmata remains the same, but the proportion of these that are terminal increases during prophase and it increases in a characteristically different way in the different types of bivalent and in those with different total numbers of chiasmata (Table III and Fig. 17). This agrees with the assumption that the terminal chiasmata arise by movement of earlier interstitial ones to the ends. Thus, amongst those with 2, 3, and 4 chiasmata, the proportion that are terminal (the *terminalization coefficient*) is the same at each stage. This indicates that originally all the chiasmata were interstitial as required by the hypothesis. Further, as in *Tulipa* (Darlington and Janaki

TABLE III

Numbers of terminal chiasmata per bivalent in bivalents with different total numbers of chiasmata (derived from Table I)

Stage	Long Type			Medium Type		Short Type
	2	3	4	1	2	1
Mid Diplotene	.33	.42	.53	.04	.58	.64
Late Diplotene	.27	.62	.64	.20	.46	.81
Diakinesis	.52	.91	1.30	.48	1.00	.90
Metaphase	.84	1.22	1.54	.49	1.00	.79

Ammal, 1932), those with one chiasma show less movement, especially in the early stages, than those with two or more. Finally, the occurrence of more terminal chiasmata at the same stages in the corresponding classes of the shorter chromosomes is to be expected on the assumption of movement since the chiasmata are more concentrated in these chromosomes and have not so far to go to reach the ends.

The only change that takes place in terminalization in the long chromosomes in this organism is the expansion of closed loops at the expense of the distal arms, a change which according to the electrostatic hypothesis (Darlington and Dark, 1932) is due to a repulsion between distributed surface charges on the paired chromatids. In the medium and short chromosomes there is also a slight movement of single chiasmata owing to the special repulsion of the spindle attachments, but this movement is ineffective in moving a proximal chiasma against the repulsion of a loop when two are present owing to the terminal spindle attachments always lying in open arms in which repulsion is less effective.

The terminalization of the chiasmata in the short chromosomes is therefore very like that in the fragments of *Fritillaria imperialis* where all chiasmata are terminalized early although only the distal chiasmata move to the end in the major chromosomes with numerous chiasmata (Darlington, 1930).

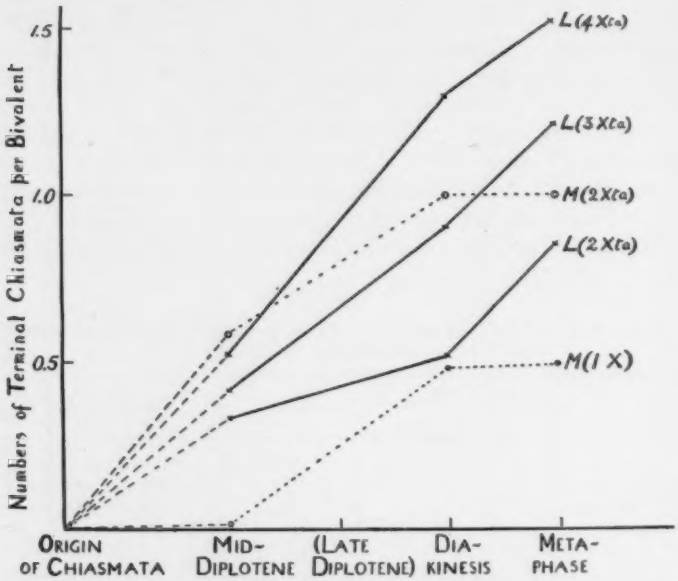


FIG. 17. Graph showing that the increase in the numbers of terminal chiasmata is in proportion to the total number at all stages where more than two are formed but not in the earlier stages where only one is formed. The short chromosome type and the late diplotene stage are omitted because the numbers of observations are smaller than those given.

SUMMARY

1. A study of meiosis in male *Chorthippus elegans*, Acrididæ ($2n = 16 + X$) shows the chromosome behaviour to be similar to that already described in *Stenobothrus parallelus*. Thus the chiasma frequency is an indirect function of length and has an interference curve of variation. Failure of pairing occurs in one chromosome type with a frequency in keeping with the curve. Terminalization depends entirely on the generalized repulsion in bivalents with closed loops and the localized spindle attachment repulsions are of the minimum degree found in *Fritillaria*.

2. A more extensive quantitative study makes it possible to show in the long chromosome type, that the number of terminal chiasmata at each stage between diplotene and metaphase is proportional to the number of interstitial chiasmata and increases from one stage to the next *pari passu* with the decrease in the number of the interstitial chiasmata. This confirms the earlier arguments that all interstitial chiasmata become terminal by movement without breakage while, on the other hand, terminal chiasmata always arise from earlier interstitial ones and in no other way. It is now clear that chiasmata always change their position after their formation at diplotene so that the configurations observed later are merely positions of changing equilibrium.

3. The opening of the diplotene loops has been followed in detail and shows that the chromosomes are single and undivided until this stage. The opening is therefore derived solely from the so-called " reductional " split, not from the " equational " one which only begins to appear at this time.

4. Syndiploidy occurs frequently just before metaphase.

For bibliography, see p. 370 of the following paper by the same author.

THE ORIGIN AND BEHAVIOUR OF CHIASMATA

VI. Hyacinthus amethystinus

C. D. DARLINGTON

John Innes Horticultural Institution, Merton, London

The original frequency and distribution and the later behaviour of chiasmata have been made clear in a number of organisms by earlier studies in this series and the comparison of observations on *Campanula, Tulipa, Fritillaria,* and *Stenobothrus* has shown us how to analyse the changes undergone by chiasmata in terminalization and has enabled us to define the forces at work in producing these changes (Darlington and Dark, 1932). Hence it is now possible to recognise from typical metaphase conditions of other organisms what the prophase conditions preceding and determining them must have been. This method can be satisfactorily applied to many species in which the prophase is not amenable to direct study.

A number of species of plants and animals are known with an extreme range in the sizes of their chromosomes (*cf.* Darlington, 1932). In species with slight size range the number of chiasmata formed is as a rule roughly proportionate to the lengths of the chromosomes at pachytene (*e.g.,* in *Hyacinthus orientalis, Fritillaria imperialis,* and *Vicia Faba*). This would only be possible with a great range of size if the longer chromosomes had a very high number, for the shortest must always form one chiasma, according to the chiasma theory of pairing, to ensure that they pair regularly (Darlington, 1930).

The indirect size-frequency relation expected on this theory has been found in *Stenobothrus* and *Chorthippus* where the extreme lengths are as 8 to 1. It may be inferred on the analogy of the observations in *Brachystola, Yucca flaccida* (O'Mara, 1931), and in the South American Acrididæ (Saez, 1930). In the last the abnormal frequency relation is evidently due to localization of chiasmata near the spindle attachment so that the same length of chromosome is concerned in forming chiasmata in all the chromosomes. In the other examples the distribution is even and the mechanism controlling the abnormal length-frequency relation cannot be so directly inferred.

High size ranges occur in many species of monocotyledons. In *Eucomis bicolor,* $2n = 32$ (Fig. 1) (*cf.* Müller, 1912), and in *Hyacin-*

thus amethystinus, $2n = 28$ [1] (Fig. 2), the range is about 20 to 1, *i.e.*, the same as in *Drosophila melanogaster*. It is to be noted that the shortest chromosomes are too small to attain the characteristic chromatid breadth of the species. In *Hyacinthus* the complement consists of 10 chromosomes about 5 μ long together with 18 less than 1 μ long and an average about one-tenth the length of the longer chromosomes.

At the first metaphase of meiosis 14 bivalents are regularly found in *Hyacinthus amethystinus*. Polar views show the five long pairs with two or three chiasmata (Fig. 3). The detailed structure of the nine short pairs cannot be determined from this aspect. As always, it is necessary to examine bivalents of this size in side view. (This is not

FIG. 1

FIG. 2

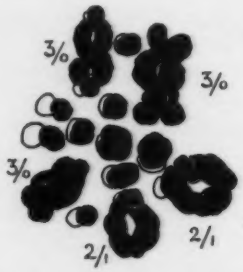
FIGS. 1 and 2. Mitotic metaphases from the root tip. × 3200.
FIG. 1. *Eucomis bicolor*, $2n = 32$.
FIG. 2. *Hyacinthus amethystinus*, $2n = 28$.

yet generally appreciated.) It is then found (Fig. 4) that most have a single terminal chiasma, a few have a single interstitial chiasma and occasionally there is one with two chiasmata. The mean chiasma frequencies of long and short types in this division are 2.4 and 1.1 respectively.

These observations show the closest analogy with *Stenobothrus* and *Chorthippus* except that the departure from normal in length-frequency relation is even more pronounced. The degree of terminalization is the same: the terminalization coefficient for the longer chromosomes is .45 in *Stenobothrus* and .42 in *Hyacinthus*, for the shorter chromosomes .67 in *Stenobothrus* and .60 in *Hyacinthus*. It is therefore evident that the conditions of terminalization are similar in the two instances and that since chiasma frequency is not reduced during prophase in *Stenobothrus*

[1] The somatic chromosome number is given by Heitz (1926) as 24.

it is similarly unaltered in *Hyacinthus*, the only change being a movement of interstitial chiasmata to the ends of the chromosomes.



FIGS. 3 and 4. First metaphase of meiosis in *Hyacinthus amethystinus*. × 3200. The total numbers of chiasmata and numbers terminal are given. Sections cut at 24 μ.

FIG. 3. Polar view. The structure is only identifiable in the long bivalents.

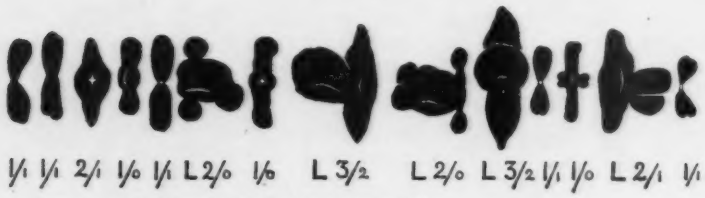


FIG. 4. Side view, bivalents drawn separately.

SUMMARY

The longer chromosomes of *Hyacinthus amethystinus* are on the average ten times the bulk of the shorter ones but have only twice as many chiasmata per bivalent. Thus, although the longer chromosomes form only two or three chiasmata, the shortest chromosomes regularly form one chiasma which ensures their regular pairing. This abnormality is characteristic of particular species and, like other variations in chiasma frequency and distribution, it must be genetically controlled. It is therefore to be regarded as an adaptive property.

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DOMINANCE OF TWO KIDNEY ALLELOMORPHS IN HABROBRACON JUGLANDIS (ASH.)

B. R. SPEICHER

(From the Zoological Laboratory, University of Pittsburgh, and the Marine Biological Laboratory, Woods Hole, Massachusetts)

INTRODUCTION

An allelomorphic series of at least four factors affecting the size of the compound eye has been located on the first chromosome of *Habrobracon*. The present paper deals with the effects of two of these. One, extreme small (k^e), was reported (Dunning, 1931) by W. F. Dunning and was kindly sent by her to P. W. Whiting. The eye size of mutant-type wasps showing this character is extremely variable, ranging from a total lack of eyes to those which, though approaching, never reach the normal size. The individual facets, when present, are of normal shape and size, the variation occurring as a decrease in the total number. The sizes of right and left eyes vary somewhat independently of each other. The ocelli are likewise affected and are also extremely variable, ranging from none at all to those apparently normal in size. Although no actual measurements have been made, it has been noted that the ocelli of any one individual tend to be of the same size. Aside from the modification of compound and simple eyes no other external effect is manifest. The mutant-type exhibits excellent viability when reared under standard conditions.

Kidney (k), an X-ray mutation found (Whiting, 1932) by Anna R. Whiting, presents superficially all of the characteristics of extreme small, but shows a high percentage of lethality when reared at standard temperature (30° C.). At lower temperatures these wasps show a viability equal to that of extreme small.

The striking similarity of the two mutants, coupled with their allelomorphism, suggested need for further study. Are they actually two separate factors having, except for the thermo-lethality of one, the same methods of expression, or is kidney a re-mutation to extreme small but carrying with it a closely linked factor causing lethality at higher temperatures? If the former hypothesis is correct, what are the conditions in regard to dominance? It was considered probable that a statistical study of the two types would bring out facts which, because of variability, would be obscure at casual inspection.

The writer is indebted to the Committee on Effects of Radiation on Living Organisms of the National Research Council for technical assistance through a grant to Dr. P. W. Whiting. Acknowledgment should also be made to Dr. Anna R. Whiting and to Dr. W. F. Dunning for making available the mutations here used, and to Dr. P. W. Whiting, at whose suggestion this work was done and whose help was greatly appreciated. The writer is likewise grateful to Miss Kathryn A. Gilmore for suggestions regarding technical difficulties.

Materials and Methods

Crosses Made

Two groups of females, one heterozygous for extreme small, the other heterozygous for kidney, were obtained by mating wild type (Stock No. 11) females to extreme small and to kidney males respectively. These heterozygous females were mated as follows:

(1) ♀ *Kk*^{e} by ♂ *k*^{e}.
(2) ♀ *Kk*^{e} by ♂ *k*.
(3) ♀ *Kk* by ♂ *k*^{e}.
(4) ♀ *Kk* by ♂ *k*.

The offspring from one half of the females of each of these four crosses were reared at 23° C. and those of the other half were reared at 30° C. All progeny were graded according to (1) the diameters of the compound eyes and (2) the size of the ocelli. Considering the number of mutant eyes counted, any measurement by facet count was impracticable. Consequently the lesser diameter of the eye was used as a basis of comparison.

Grading of Compound Eyes and Ocelli

Six grades of eye size were arbitrarily marked off (Fig. 1), of which four were set off in the following manner. From a stock of mutant-type wasps the individual possessing the largest eye was selected. With this eye as the upper limit of variability and the bare indication of the scleral ring surrounding the eye as the lower limit, four grades were established covering equal ranges in regard to eye diameter. These groups constituted Grades 1, 2, 3, and 4, in increasing order of size. During the process of grading the experimental material, a few eyes were found which exceeded the upper limits of Grade 4; these were placed in a separate group, Grade 5. Although, theoretically, Grade 5 extends from Grade 4 to wild-type, in no case did the sizes of any eyes belonging to this grade so closely approach that of type as to cause the two to be indistinguishable. It was considered advantageous to record separately the eyes which had failed to develop;

this group was designated Grade 0. Thus Grades 0 and 5 mark the extremes in variation of eye size with Grades 1, 2, 3, and 4 representing the intermediate stages.

Eyes were selected (to be used as standards of comparison with the experimental material) which met the theoretical specifications of the grades. As an aid to more accurate grading, each standard was made to represent the upper limit of its grade. Eyes under question as to their proper position in the series could then be compared with the standard most nearly resembling them and if of the same size or slightly smaller would be placed in the same grade as

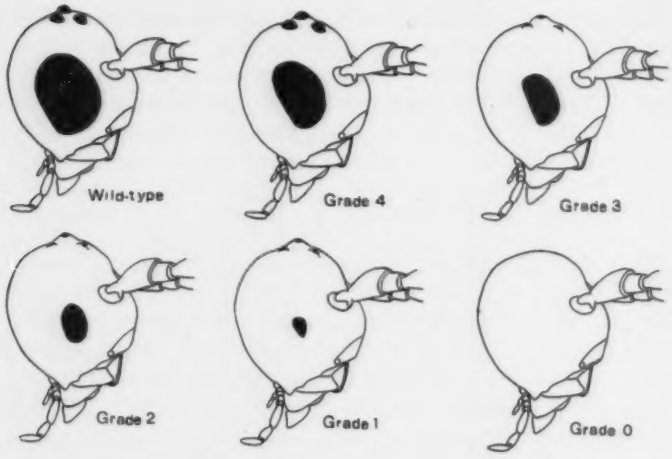

FIG. 1. Eyes of wild and mutant-type wasps. Illustrations of mutant-type wasps represent the upper limits of their designated grades. × 33.

represented by the standard, but if they were larger they would be classified as belonging to the next higher grade. Each wasp was compared directly with these standards, the sizes of right and left eyes being recorded separately.

The ocelli were divided into three groups, absent, small, and normal. Although, as mentioned above, the ocelli of any one individual tended to be of the same size, in case of disparity the wasp was classified in the small ocelli group.

Recording of Lethal Wasps

In *Habrobracon* lethal individuals may be recognized by the presence of shriveled larvæ or blackened pupæ still confined within the

cocoons. While lethality occurring during the development of the egg may escape detection, some estimation of the number of lethal wasps may be made by scrutinizing the culture vials. Lethal counts given in this paper were made by using the above method.

Presentation of Data

Comparative Lethality of Mutant Types

Among the four classes of wasps which were reared at 30°, two had an excessive number of lethal individuals. As kidney is known to be lethal when reared at this temperature, the fraternities containing kidney wasps may be expected to be the ones most affected. As seen in Table I, offspring of the crosses $Kk \times k^e$ and $Kk \times k$ contain

Table I

Numbers of type, mutant, and lethal wasps resulting from different crosses reared at different temperatures.

Origin	Wasps reared at 30° C.				Wasps reared at 23° C.			
	$Kk^e \times k^e$	$Kk^e \times k$	$Kk \times k^e$	$Kk \times k$	$Kk^e \times k^e$	$Kk^e \times k$	$Kk \times k^e$	$Kk \times k$
Type.....	301	432	344	198	222	275	239	182
Mutant.....	300	383	172	23	220	232	240	178
Lethal.....	71	44	119	134	47	54	33	45

by far the greater number of lethal wasps. The cross $Kk \times k^e$ produces kidney males and the cross $Kk \times k$ produces both kidney males and kidney females, thus accounting for the greater number of lethal individuals among the progeny of the former cross and the still greater number among those of the latter cross.

The number of males produced in proportion to the females is an important factor affecting the comparative lethal ratios. An excess of males in a class coming from the cross $Kk \times k^e$ would raise the entire lethal ratio and, since the males of the lethal group were not separated from the females, the cause for the increase would not readily be apparent. In this case the sex ratio of the mutant-type must be considered, and may be assumed to be approximately the same as that of the type wasps of the same group. In the cross $Kk \times k^e$, reared at 30°, there was an excess of males, but not of statistical significance. In the other groups reared at 30° and 23° the sex ratios were likewise not significantly different.

Offspring from the cross $Kk^e \times k$ had a low lethal ratio. The mutant-type females of this group, being extreme small-kidney compounds, should demonstrate the dominance of one or the other factor

in regard to thermo-lethality. The low lethal ratio and the abundance of viable compound females (Table I) indicate that the thermo-lethal trait associated with kidney is recessive. The lethal ratios of the four classes reared at 23° were not significantly different, since neither mutant factor causes an excess of lethals at this temperature.

A peculiarity brought out in Table I which is worthy of passing notice is the excessive number of mutant-type wasps in classes reared at both temperatures. Since the number of lethals counted represents the minimum actually produced, and the major part of the lethal group consists of mutant-type wasps, this excess is obvious, although the cause is as yet unexplained.

TABLE II

Grades of eyes and of ocelli tabulated according to genetic constitution and temperature. As regards eye size, the numbers in the body of the table indicate numbers of eyes graded. As regards grades of ocelli, the numbers indicate individuals.

	Wasps reared at 30° C.					Wasps reared at 23° C.				
	k^s	k^sk^s	k^sk	kk	k	k^s	k^sk^s	k^sk	kk	k
Eye size										
Grade 0	86	97	305	7	33	6	39			
1	83	51	120	4	11	19	38			
2	300	119	308	11	8	109	113	2	1	
3	115	23	55		4	84	71	41	6	6
4	14	2				56	11	624	193	308
5								3	2	6
Mean	1.81	1.25	1.14	0.68	0.64	2.60	1.92	3.94	3.97	4.00
Ocelli										
Lacking	1	4	19	10	13		2			
Small	289	141	374	1	15	136	134	320	10	5
Normal	9	1	1			1		15	91	156

Comparative Eye Sizes of Mutant Types

The effect of a 30° temperature on the eye sizes of the various genetic classes is seen in Table II. Most of the offspring from each of the four types of matings possess eyes which fall into the lower groups, Grades 2 and 0 being particularly well represented. The fact is well demonstrated in the classes unaffected by thermo-lethality and is suggested in the kidney classes, although in these latter groups the numbers are not sufficient to be statistically significant.

The dispersal of wasps according to the sizes of both right and left eyes is seen in Table III. Among the wasps reared at 30° the groups composed of extreme small females, compound extreme small-kidney females, and to a lesser extent the extreme small males show a preponderance of individuals in four places, involving Grades 0 and 2.

TABLE III

Correlation tables showing the relation of size of right and left eyes in wasps of different genetic constitution reared at different temperatures. Ordinates represent grades of left eyes and abscissæ represent grades of right eyes. The numbers in the body of the table indicate the number of individuals reared, the eyes of which fall in the designated grades.

		30°						23°					
		0	1	2	3	4	5	0	1	2	3	4	5
♂,k°	5												
	4			7	1	1				5	10	17	
	3	5	7	24	19				1	12	17	3	
	2	12	15	84	35	1		3	5	29	22	3	
	1	11	9	22	2	2			3	2	4	1	
	0	16	5	16	4	1							
♀,k°k°	5												
	4		1					2	1	1		1	
	3	4	3	4	2				8	16	13	2	
	2	22	13	27	3	1		5	4	28	13	2	
	1	13	5	6	3			2	2	12	2		
	0	19	3	16	1			8	5	4	4	1	
♀,k°k	5											2	
	4									1	10	296	1
	3	5	4	15	6						7	17	
	2	50	18	78	5							1	
	1	18	14	19	5								
	0	78	28	45	6								
♀,kk	5												1
	4										1	95	
	3										2	1	
	2	3	1	3								1	
	1	1		1									
	0	1	1										
♂,k	5												1
	4										1	151	3
	3				2						1	3	
	2	3	0	1									
	1	4	2										
	0	1	3	1									

Thus in the compound extreme small-kidney group in Table III, the areas 0, 0 (78 individuals), 0, 2 (50 individuals), 2, 0 (45 individuals), and 2, 2 (78 individuals) possess an abundance of wasps as compared with the surrounding squares. This indicates a tendency for the wasps to have the eyes either entirely lacking or developed up to a stage represented by Grade 2, which is apparently somewhat of a limit for eye size of wasps reared at this temperature.

The bimodality of each of these classes, as represented in Tables II and III, may be due in part to the method of measurement. The anatomical variation of the eye, although used as a basis of comparison, is a secondary result of a more fundamental but unmeasured physiological change within the organism. In Grade 0 this variation reaches a lower limit of expression. The physiological variation, however, does not stop here but grades down to the lethal point. Below the threshold of viability the effects are again perceptible externally, this time as lethals, the majority of which have the eyes entirely lacking and are noticeably micro-cephalic. There is, then, a range of physiological variation which can be seen externally only by Grade 0 and the lethal group, and as these two groups must contain those individuals falling into the intermediate physiological grades their numbers are thus increased. From this it appears that Grade 0 is not a true mode of eye size, the strong representation of individuals in this grade being a result of the methods used in calibrating the variation. The true mode is confined to the upper limit of variation, which for those classes reared at 30° centers around Grade 2. This explanation, although difficult to prove, is supported by the fact that in the arrays of eye sizes of wasps reared at 23° (in which cases the ranges of physiological variation have been raised) there is but one mode present, centering around the upper limits of variation.

A difference in the expression of the mutant factors appears in the classes reared at 23°. As seen in Tables II and III, extreme small wasps possess eyes which cover the same grades as do those of the corresponding group reared at 30°, although their means (Table II) are slightly higher. In contrast to this is the array of kidney wasps which at 23° have the eyes concentrated around a single high grade. The compound extreme small-kidney females follow the kidney trait in the concentration of eye size around Grade 4, indicating a complete dominance of kidney over extreme small in regard to this character.

A comparison of means of eye size is given in Table II. The difference between the means of extreme small and kidney wasps, both reared at 30°, suggests a difference caused by the two factors at this temperature, although the numbers are too small to be statistically significant.

In judging the variation of the ocelli of wasps reared at 30° the modes of the classes containing the factor extreme small are in the "small" group, as represented in Table II. In the kidney wasps the mode appears to have shifted down towards the "lacking" group, although here again the small number of viable wasps is not sufficient to be of any statistical significance. In the 23° groups, however, the numbers are large enough to make comparisons. At this temperature the extreme small groups retain their small ocelli, but in the kidney groups the majority of ocelli appear normal in size. The compound extreme small-kidney females resemble the extreme small group at this temperature in regard to the size of the ocelli.

Correlation in Size of Right and Left Eyes

At 23° kidney and kidney-extreme small compounds showed little variation in regard to eye size, and at 30° kidney wasps were too few to give significant results. Correlation coefficients were calculated as follows:

Extreme small males, reared at 30°,	$r = 0.2939 \pm 0.0352$.
Extreme small females, reared at 30°,	$r = 0.1129 \pm 0.0568$.
Extreme small-kidney females reared at 30°,	$r = 0.2375 \pm 0.0353$.
Extreme small males reared at 23°,	$r = 0.4520 \pm 0.0458$.
Extreme small females reared at 23°,	$r = 0.2351 \pm 0.0546$.

It is evident that correlation in eye size exists although it appears to be not very high under the conditions of measurement.

Summary and Conclusions

1. Two mutant allelomorphs in *Habrobracon*, extreme small, k^e, and kidney, k, superficially resembling each other in their effects on eye size, were studied to determine their interrelationships.

2. Kidney wasps reared at 30° are for the most lethal, appearing in general as eyeless small-headed pupæ. Extreme small are viable at 30° and no lethal effect is evident at 23° in either type. Extreme small is dominant as regards thermo-lethality since extreme small-kidney compound females are of normal viability when reared at 30°.

3. In extreme small wasps size of compound eye is relatively little affected by temperature. In kidney, however, a lower temperature causes a much larger size. Kidney is dominant in this respect in the compound females.

4. In extreme small wasps size of ocelli is likewise relatively little affected by temperature, being "small" both at 30° and at 23°. In kidney, however, the majority reared at 23° have normal ocelli, while

at 30° ocelli are smaller or altogether lacking. Extreme small is dominant over kidney as to ocellar size both at 30° and at 23°.

5. There is a decided tendency in both mutant types for the compound eye either to be entirely absent or to develop to a certain modal size determined by temperature and genetic constitution.

6. There is a low but significant correlation as to size between right and left eyes.

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THE MOSAIC DEVELOPMENT OF THE ASCIDIAN EGG

N. J. BERRILL

DEPARTMENT OF ZOÖLOGY, MCGILL UNIVERSITY, MONTREAL

The development of ascidian eggs has long been considered, as the result of the classical investigations by Conklin, to be the perfect example of the mosaic rather than the regulative type.

Different blastomeres of dividing eggs were killed or sufficiently injured to inhibit further development and the development of the remaining blastomere or blastomeres was followed. He found that each blastomere developed only into the tissues and organs that it would have produced had it remained a part of intact egg or embryo. In no case did a whole but dwarf larva result from one of the first two or four cells. The left blastomere of the 2-cell stage gave rise to the organs of the left side only. The anterior two blastomeres of the 4-cell stage gave rise to epidermal, neural, and chordal tissues, but failed either to gastrulate or to form a tail.

In general the organization of amphibian eggs is similar to that of ascidian eggs, formative substances appearing rather later, although Hall has recently shown that they are delimited as early as the 8-cell stage. Isolated blastomeres of the 2-cell stage, however, may give rise either to a whole larva of half the normal size or to a right or left half-larva of normal size, according to whether or not there has been reorganization of the cell contents.

In consequence of this it has seemed possible that the absence of regulation or reorganization in Conklin's experiments may have been due to the fact that the injured blastomeres were left in situ within the egg membrane. The presence of their inert mass in contact with the surviving part may have had an inhibitory effect. This possibility has been emphasized by Reverberi's conclusions, based upon experiments with *Ciona* eggs, that the eggs of ascidians should be placed properly within the regulative class.

The great obstacle to experimental work with ascidian eggs has been the difficulty of removing the egg membranes and follicle cells without injuring the ovum. It is partly the object of this paper to record a method by which this may be done. Several ascidian eggs are shown in Fig. 1, and it is seen that there is considerable variation in the character of these membranes.

It has already been shown (Berrill, 1929) that the membranes are normally digested from within by a proteolytic enzyme secreted by the developing tadpole, that the enzyme is fairly stable and is active within the limits pH 7.0–10.0. Trypsin digests the membranes, but only very slowly, so that the protease concerned is probably a tryptic ferment of a less specialized nature than trypsin itself.

There are, accordingly, two means by which membranes of ascidian eggs may be removed. The enzyme produced by developing ascidian tadpoles may be collected and concentrated, or a suitable and more readily obtainable substitute may be found. It was discovered that *Ciona*, *Phallusia*, *Ascidia*, and *Ascidiella* eggs remained about 100 per cent viable at 18° C. for a little over twenty-four hours, so that any enzyme mixture to be of use must be effective within that period.

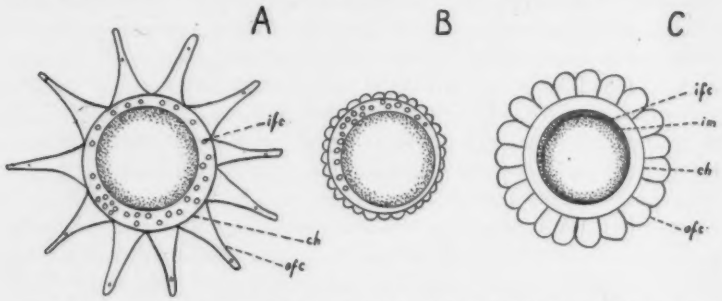

FIG. 1. Ascidian eggs. *A*, *Ciona intestinalis; B*, *Molgula* or *Polycarpa; C*, *Ascidiella aspersa*. *ifc*, inner follicle cell; *im*, inner membrane; *ch*, chorion or egg-membrane; *ofc*, outer follicle cell.

The natural ascidian enzyme in sufficient concentration is preferable to any substitute, but it can be obtained only from species of the family Ascidiidae (i.e., the genera *Ascidia*, *Ascidiella*, *Phallusia*) and *Ciona*. To obtain it in effective concentration it is necessary to make mass cultures of some thousands of embryos. Normal development of large numbers of eggs necessitates the use of a relatively large volume of water, the larger the better, for it is only when development has proceeded to within two or three hours of hatching that concentration becomes effective. Then the embryos are allowed to settle on the bottom of the vessels and as much supernatant water as possible is poured off. The remainder, together with the embryos, is poured into a test tube or cylinder, the embryos again allowed to settle, and the water reduced to about 5 cc. This with the embryos is then spread over the bottom of a Petri dish to ensure a supply of oxygen to the embryos, left there until hatching is complete, and the water then

collected. It should contain enough enzyme to digest off the membranes from unfertilized *Ciona* or *Ascidia* eggs in from five to ten hours. Such eggs may subsequently be fertilized.

If it is desired to remove the membranes from merely a small number of eggs, the eggs may be placed among the developing tadpoles about three hours before they are due to hatch, when their membranes will dissolve about an hour after hatching (the developing tadpoles should be close enough together to be almost touching and to form a sheet one layer deep). It may be desirable to remove the layer of outer follicle cells by washing the eggs in a bag of fine bolting silk, when the enzyme can more readily attack the underlying membrane.

Large *Ascidia* or *Ciona* are not, however, always obtainable, and it is often more convenient to use a substitute for the ascidian enzyme. Such substitutes may be obtained from any large carnivorous or omnivorous invertebrate, the most active mixtures being the stomach juices themselves rather than gland extracts or preparations.

Those actually used were the clear juices found in the stomach of decapod crustacea such as *Munida*, *Maia*, and *Homarus*. No doubt other forms would be equally effective.

Undiluted, such juices are too potent and toxic, but a dilution of one part juice with fifty or one hundred parts of sea-water is both safe and efficient. The time taken for the membranes to be removed depends not only upon the activity of the enzyme but also on the thickness of the membrane and on the temperature. At 18° C. the membranes of *Ciona*, *Molgula*, or *Ascidia* are removed in from two to four hours, but the thicker membranes of *Polycarpa* or *Styela* require more than twenty-four hours. Unfertilized eggs of *Ciona*, *Molgula*, and *Ascidia* that have had their membranes removed in this way may subsequently be fertilized and undergo normal development.

If fertilized eggs are subjected to such a mixture or if eggs are fertilized while yet within it, a somewhat unexpected feature appears. Nuclear division proceeds normally, at least to the 32-cell stage, but cytoplasmic division is inhibited. In the case of later embryos, the surface cells round off and tend to fall away. These phenomena are apparently due to surface action of the lipochrome contained in the original digestion mixture, and in consequence this method of removing the membranes is satisfactory only in the case of unfertilized eggs. In this last case the capacity for fertilization and normal development is unaffected.

Once membranes have been removed, experiments of two kinds become possible. The naked eggs may be cut with glass needles, as

used by Horstädius (1928), while blastomeres may be separated from one another. Attempts to cut the eggs were made only in the cases of *Ascidia* and *Ciona*. Those of *Ascidia* (*Ascidiella aspersa*) possessed a tough surface and a very fluid endoplasm, so that all attempts at cutting resulted in bursting them. The eggs of *Ciona*, on the other hand, are readily cut but possess such a viscous endoplasm that the injured surface does not re-form. In neither case, therefore, is experimental work of this nature feasible. It is just possible, however, that other eggs, such as those of *Molgula* or of other species of *Ascidia*, may be more suitable and of a type intermediate between those of *Ciona* and *Ascidiella*.

Separation of blastomeres, on the other hand, is very easy to effect. They may be separated with a glass needle, as used by Horstädius, or more simply merely by slight shaking. Pouring water in which there may be some hundreds of naked eggs in the 2-cell or in the 4-cell stage from one vessel to another several times results usually in the complete separation from one another of all blastomeres. There is a tendency among the blastomeres of *Ciona* eggs to fall apart in any case, though eggs and early embryos of *Ascidiella* tend to fuse on contact.

In the subsequent development of blastomeres isolated in the above manner no indication was found of any reorganization. Blastomeres isolated in the 2-cell stage invariably give rise to lateral half-embryos that gastrulate imperfectly and have but twenty instead of forty notochordal cells which, moreover, fail to interdigitate. Anterior blastomeres isolated at the 4-cell stage formed small spheres of ectoderm with about ten notochord cells loosely connected with the outer surface. Posterior blastomeres derived from the same stage formed small spheres of endoderm and mesoderm enclosed within ectodermal cells. Illustrations of such partial development are shown in Fig. 2. Each isolated blastomere apparently divides to form just those cells and tissues it would have formed had it remained a part of a whole embryo. Never was there any sign of development to form a whole larva of half or quarter normal size.

It is concluded, therefore, that Conklin's results are in no way invalidated by his failure to remove the dead or injured blastomeres or the egg membrane, and his conclusions concerning the mosaic nature of the ascidian egg are confirmed.

Reverberi, on the other hand, has reached conclusions of a very different nature. By an ingenious method of puncturing the chorion and compressing the ovum he succeeded in dividing *Ciona* eggs into two unequal parts, both parts subsequently being fertilized. His main conclusion, that the ascidian egg should definitely be assigned

to the regulative class, is based upon two discoveries. The segmentation of the small extruded part of the ovum not only commences in a plane at various angles to the first cleavage plane of the ovum proper, but proceeds in a fairly typical manner; while eggs part of which have been extruded are often able to develop to form an apparently perfect tadpole larva.

These statements are not questioned, but they are by no means incompatible with the mosaic conception.

The development of the small extruded part is normal only in so far as the first three cleavages are in the three planes of space as in the normal egg. Gastrulation and coördinated development do not occur. Since, with the exception of the Nematelminthes, the seg-

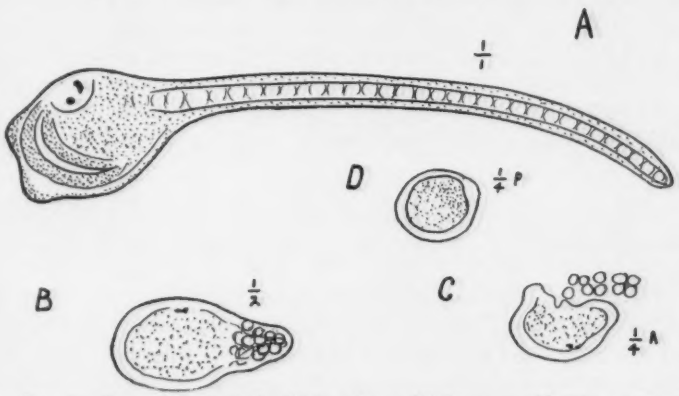

FIG. 2. Development of *Ascidiella aspersa* (24 hours at 17° C.). *A*, normal tadpole; *B*, development of isolated blastomere of 2-cell stage; *C*, of anterior blastomere of 4-cell stage; *D*, of posterior blastomere of 4-cell stage.

mentation of almost all animal eggs is uniform, the first cleavage being vertical, the second vertical at right angles to the first, and the third horizontal, the ability of part of an ascidian egg to follow this same course is alone hardly evidence of strict regulation and any other sequence would be startling. The outstanding exception to such a course, namely the segmentation of the egg of *Ascaris*, results in a very unstable configuration. The spindle tends to lie along the longest cytoplasmic axis, and the fact that a fertilized part of an ascidian egg obeys this rule of Hertwig in the first stages merely emphasizes its validity. It has no real bearing upon the mosaic or regulative nature of the egg.

The other experiments supposedly showing a regulative capacity on the part of the egg concerned the development of diploid eggs,

part of which had been extruded after fertilization. These developed to form apparently normal tadpoles of reduced size. In no case was it found possible to orientate the eggs with regard to their oöplasmic contents. The only illustrations, however, show two larvæ with tails about four-fifths full length and with a trunk region from about four-fifths normal to full size. The one with the smaller trunk has the longer tail, and if the part extruded consisted of part of the endodermal region of the ovum, the peculiarity of this tadpole is accounted for. Similarly, the large-bodied, short-tailed larva is explained if the part extruded contained more of the chordal crescent and less of the endodermal oöplasmic region. No growth in size, other than the swelling of notochord cells, occurs in *Ciona* until after the tadpole stage has been passed, so that the size of the tadpole is a fairly accurate indication of the size of the egg, and it is evident in these two cases that only a very small part can have been extruded. In any case the peculiarities of the two examples illustrated can be as readily explained on the basis of the mosaic conception as on the assumption that the egg is more regulative than is generally believed.

Summary

A new method of removing the membranes from ascidian eggs in the mass is described. Essentially the method is the use of proteolytic enzymes.

The development of isolated blastomeres is described, confirming Conklin's results that the development of each is strictly partial and that the whole development is a mosaic. It is also shown that the presence of the egg membrane and of injured or dead blastomeres in his experiments in no way invalidated those results.

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THE EARLY EMBRYOLOGY OF THE ECHIUROID, URECHIS

W. W. NEWBY

DEPARTMENT OF ZOÖLOGY, UNIVERSITY OF UTAH

INTRODUCTION

The eggs of *Urechis* are being used for many investigations in experimental embryology and a need for a study of the normal development has been felt. The object of the present investigation has been: first, to develop a technique whereby either normal or experimentally treated eggs may be stained and preserved for permanent record; second, to describe and figure the early stages of the development; and third, to compare the development of *Urechis* with that of *Thalassema mellita* (Conn), as described by Torrey, 1903.

This paper is a preliminary report, preparations for the further study of the development of the worm having been made. The work was begun in the summer of 1931, at the Hopkins Marine Station at Pacific Grove, California. During the following winter it was continued in the Zoölogical Laboratory of the University of Utah. It is with pleasure that the writer expresses his appreciation for the aid given him by Professor G. E. MacGinitie, of Hopkins Marine Station, in the collecting of the worms and the rearing of the larvæ, and his indebtedness to Dr. Harold Heath, under whose direction the work was carried on.

MATERIAL AND METHODS

The worm *Urechis caupo* was described by Fisher and MacGinitie in 1928. It is an inhabitant of the mud flats of the bays and sloughs along the California coast and dwells in U-shaped, tubular burrows. The worms used in this work were obtained from Elkhorn Slough at Monterey Bay and from Morro Bay. They were brought to the laboratory in glass jars cooled by ice packs and in the laboratory were kept in well-aërated aquaria.

The sexes are separate in *Urechis*, the eggs and sperm being stored in the six segmental organs, from which they may be easily obtained by gently probing into the ducts of the organs with a thin, smooth, glass probe. The eggs were placed in finger bowls of fresh, filtered sea water with the water about an inch deep. Enough eggs were placed in each bowl to cover the bottom with a single layer. The sperm placed in a watch-glass made a rather thick fluid. This was diluted with about ten volumes of sea water. Insemination was

effected by the addition of three drops of the sperm dilution to each bowl of eggs. This produced almost 100 per cent fertilization with only an occasional case of polyspermy. The developing eggs were kept in a poorly lighted basement room where the temperature was nearly constant at about 20° C. At this temperature the rate of development was very constant for all the eggs, being remarkably uniform after the 16-cell stage.

The formation of the fertilization membrane, the polar bodies, and the first cleavage could be readily observed and samples of the eggs were fixed at each of these stages. After the 2-cell stage samples of the eggs were fixed every fifteen minutes.

The eggs were removed with about 2 cc. of the sea water and placed in a vial. They were fixed by the addition, drop by drop, of an equal amount of corrosive sublimate and acetic acid fixative (Guyer No. 18). After ten minutes the fluid was removed with a pipette and the eggs were washed with distilled water for two minutes. This was removed and washing was continued in 50 per cent and then in 70 per cent alcohol for two days. This treatment resulted in a shrinkage of less than 12 per cent of the diameter of the egg.

The eggs were stained for one hour and forty minutes in Mallory's phosphotungstic acid hematoxylin (Guyer No. 62). The stain was diluted to about 20 per cent and when used at this concentration, spindles of dividing cells, chromosomes, and cell boundaries were distinct. Little destaining was needed with this stain but when necessary it was done in a weak alkaline alcohol, the treatment being continued until the eggs were a light pink when observed with the low power of the microscope and with reflected light. The eggs were then dehydrated, cleared, and mounted in balsam. In all processes after the washing the eggs were not handled, but were placed in a small dish and all fluids were added or withdrawn with a pipette. The balsam was added after most of the xylene had been removed and small drops of this balsam with the eggs were easily mounted.

Contrary to a common opinion, the technique of staining and mounting is not laborious and the writer suggests that often much might be learned if experimentally treated eggs were thus preserved for closer examination.

Fertilization and the First Cleavage

The process of fertilization has been described by Tyler (1931). The process may be outlined as follows: first, the attachment of the sperm; second, the appearance of a cone on the surface of the egg immediately below the point of attachment; third, the fertilization

membrane appears on the surface of the egg and begins to lift; fourth, the sperm entrance cone of the egg begins to enlarge and the indenture of the egg begins to round out; fifth, the sperm head enters the egg. This process takes six and a half or seven minutes at 20° C. At a time about twelve minutes after the sperm attachment the nucleolus of the egg disappears and at fifteen minutes the nucleus or germinal vesicle disappears. At about thirty to thirty-five minutes the first polar body is extruded and about ten minutes later the second is extruded (Plate I, Fig. *B*). In many cases the first polar body divides so that three appear. About one hour and ten minutes after sperm attachment the egg elongates and the furrow of the first cleavage appears and by one hour and twenty minutes the egg is in the two-cell stage. The second, third and fourth cleavages take place at half-hour intervals. This outline was made from the work of Tyler, confirmed by the writer's observations on several groups of eggs at 20° C.

The unfertilized egg appears to have a marked polarity as the bottom of the indenture seems to mark one pole (Plate I, Fig. *A*). Tyler considers this as the animal pole, for in 85 per cent of the eggs observed by him the polar bodies appear at a point less than 10° from the egg surface, which before fertilization was at the bottom of the indenture. Taylor (1931) however, states: "The immature egg apparently has no mark of polarity. Neither the eccentricity of the germinal vesicle nor the position of the one or more depressions could be correlated with the subsequent place of extrusion of the polar bodies, as will be stated in some detail in later paragraphs."

The position of the axis of the spindle of the first cleavage and therefore the plane of the cleavage is determined by the axis of the egg and some other point. Tyler has found that the plane of the first cleavage passes within ten degrees of the point of entrance of the sperm in 71 per cent of 178 eggs observed. The high correlation between point of sperm entrance and the position of the first cleavage furrow is confirmed by the work of Taylor.

Development to the Sixty-Four-Cell Stage

From the two-cell to the sixteen-cell stage.—The cleavage furrow first appears at the animal pole of the egg and spreads to the vegetal pole, cutting the egg into two equal-sized cells. These are at first almost spherical, being only slightly flattened on their adjacent sides. During the resting stage they further flatten and elongate and at this time the spindles of the second cleavage may be seen (Plate I, Fig. *C*). The axes of the spindles are parallel, and in stained preparations the chromosomes may be indistinctly seen and around the ends of the spindles red granules are visible.

The second cleavage furrow also starts at the animal pole of each cell and then divides it into two. The egg is then in the 4-cell stage, the cells being equal in size and flattened on their adjacent sides (Plate I, Fig. *D*). The long axes of the cells are parallel with the axis of the egg and a distinct, equal-sided segmentation cavity lies between them. In *Thalassema*, Torrey describes the two spindles of the second cleavage as being not quite parallel so that the *A* and *C* cells are in contact at the upper pole and the *B* and *D* cells are in contact at the lower pole, the furrow at the upper pole being a segment of the first cleavage furrow. This method of cleavage was not observed either in the living or preserved eggs of *Urechis*.

Preceding the third cleavage the four cells elongate vertically and become somewhat more rounded horizontally so that the area of contact between the cells decreases. As the spindles form with their axes coinciding with the long axes of the cells, the upper ends of the cells rotate to the right, clockwise; and the lower ends rotate to the left, counter-clockwise (Plate I, Fig. *E*). The resulting cleavage is thus spiral and dexiotropic. The upper moiety is the first quartet of micromeres and the cells are slightly smaller than the lower macromeres (Plate I, Fig. *F*). There is a rather brief resting period during which the cells become flattened on their adjacent sides and the whole egg becomes more spherical.

Both the macromeres and micromeres then elongate, and their upper ends rotate to the left, counter-clockwise (Plate II, Fig. *G*). The resulting cleavage is therefore spiral and leiotropic and is somewhat unequal. The first quartet now consists of eight cells, the lower cells, $1a^2$, etc., being somewhat smaller than the parent cells and the macromeres having divided to produce the second quartet, $2a$, etc., the cells of which are the same size as the daughter cells of the first

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PLATE I

All drawings were made with a camera lucida and have the same magnification. Figures *A* and *B* were drawn from living eggs. All other figures were drawn from fixed material and are therefore somewhat smaller.

FIG. *A*. The unfertilized egg showing the indenture or depression.

FIG. *B*. The fertilized egg with the polar bodies extruded, the fertilization membrane not being shown.

FIG. *C*. A semi-polar view of the 2-cell stage showing the parallel spindles of the second cleavage.

FIG. *D*. A polar view of the 4-cell stage showing the equal-sized cells and the equal-sided segmentation cavity.

FIG. *E*. A lateral view of the 4-cell stage as the cells divide to form the 8-cell stage.

FIG. *F*. A lateral view of the 8-cell stage.

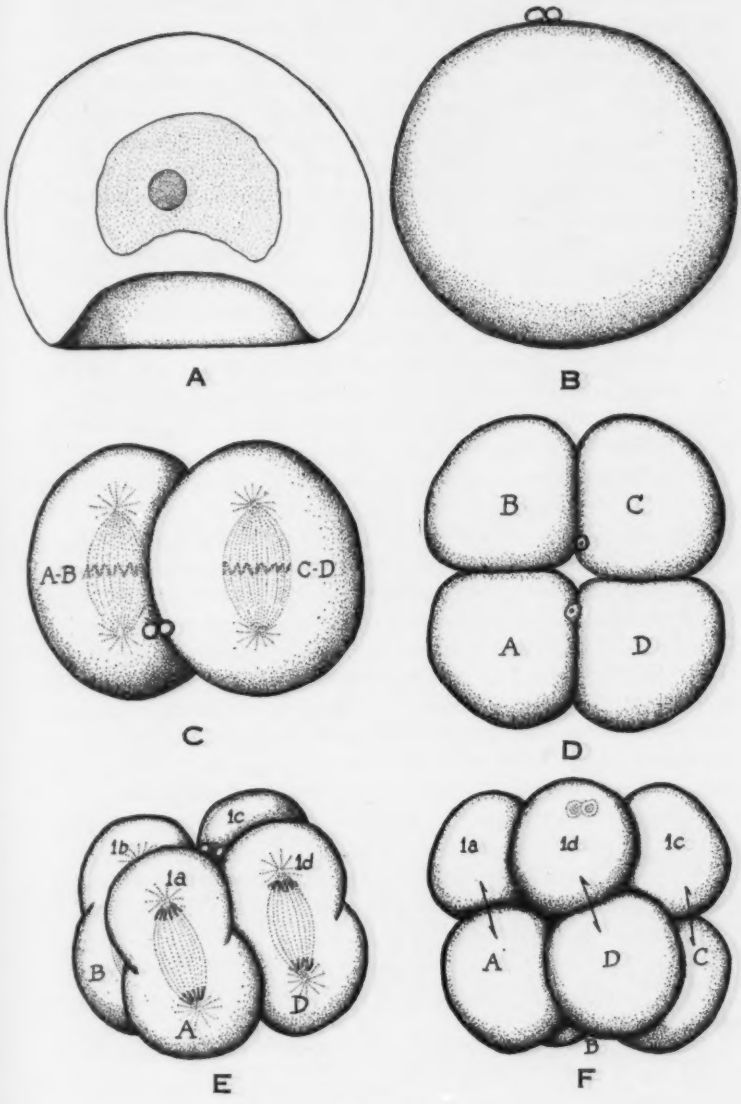

PLATE I

quartet (Plate II, Figs. *H*, *I*, and *J*). The daughter cells of the first quartet are the prototroch cells. The egg is radially symmetrical and during a rather protracted resting stage the cells become flattened on their adjacent sides. In the 8-cell stage the first quartet cells are to the right of the macromeres. In the 16-cell stage, however, because of the pressure of the newly-formed cells, the stem cells of the first quartet are pushed to the left of the macromeres.

The twenty-four-cell stage.—The next division is unequal and takes place simultaneously in the stem cells of the first quartet and in the macromeres (Plate II, Figs. *K* and *L*). It is dexiotropic and results in the formation of eight new cells, all markedly smaller than the parent cells and slightly smaller than the daughter cells of the preceding division. The egg is now in the 24-cell stage and consists of the first quartet of twelve cells, four newly formed ($1a^{1\cdot2}$, $1b^{1\cdot2}$, etc.), the second quartet of four cells, the newly-formed third quartet of four cells (*3a*, *3b*, etc.), and the four macromeres. During the rather brief resting stage the cells again flatten on their adjacent sides and by their pressure again push the stem cells of the first quartet to the right of the corresponding macromeres.

The thirty-two-cell stage.—This stage is produced by a division of the prototroch cells, $1a^2$, $1b^2$, etc., and the second quartet cells, *2a*, *2b*, etc. Both divisions are dexiotropic and about equal, but the division of the prototroch cells precedes that of the second quartet cells, the first being in a late stage of division when the latter is in an early stage (Plate III, Fig. *M*). In the resting period following this division the egg undergoes a change in shape. The cells of the first and second quartets become somewhat flattened and surround an enlarged segmentation cavity. The four macromeres remain in contact and have their adjacent sides almost vertical, the whole egg taking on a "mushroom shape" with the four macromeres appearing as four conspicuous cells at the base (Plate III, Figs. *N* and *O*).

This change in shape marks the second conspicuous deviation in the development of *Urechis* as compared with that of *Thalassema* as

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PLATE II

FIG. *G*. A lateral view of the 8-cell stage as the cells divide to form the 16-cell stage.

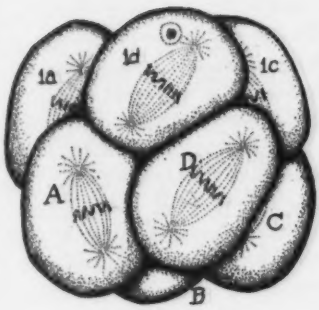
FIG. *H*. A lateral view of the early 16-cell stage.

FIG. *I*. A polar view of the early 16-cell stage.

FIG. *J*. A lateral view of the 16-cell stage during the resting period. The extent of the segmentation cavity is indicated by the dotted line.

FIG. *K*. A lateral view of the 16-cell stage as the cells divide to form the 24-cell stage.

FIG. *L*. A lateral view of the 24-cell stage.



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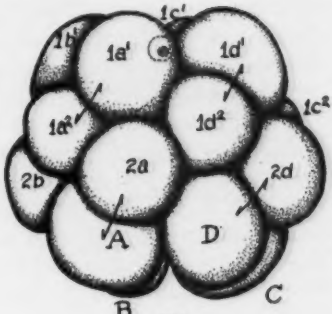

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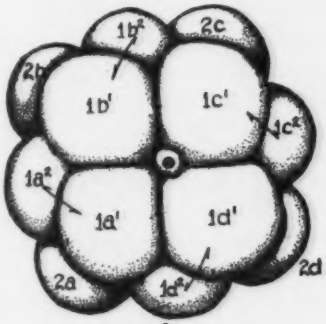

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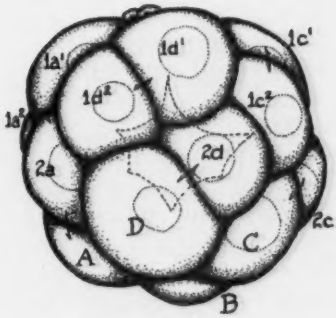

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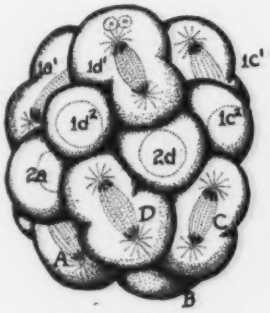

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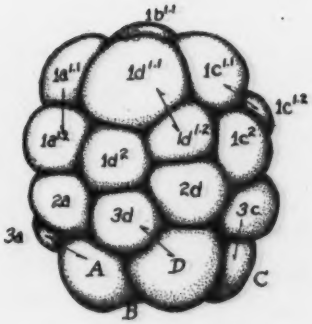


L

PLATE II

described by Torrey. The order of the cleavages has been the same and the general arrangement of the cells has been the same, but he figures the macromeres as being proportionally larger and he neither mentions nor figures the "mushroom shape" which characterizes the *Urechis* egg from this stage on to the rotating larval stage.

The thirty-six-cell stage.—This stage is formed by a leiotropic division of the macromeres to form the fourth quartet (Plate III, Figs. *N* and *O*). This division is unequal, the fourth quartet cells being even smaller than the cells of the second and third quartets. They are also pushed inward and flattened so that they form long cells extending radially into the egg and presenting a small surface to the outside.

This division is similar to that of *Thalassema* in that there is nothing in either the time of cleavage, the shape, size, or positions of the cells to distinguish the 4*d* or *M* cell from the other fourth quartet cells.

The forty-cell stage.—This stage is formed by a leiotropic division of the stem cells of the first quartet, $1a^{1.1}$, $1b^{1.1}$, etc. (Plate III, Figs. *P* and *Q*). The division is very unequal and the smaller polar cells, $1a^{1.1.1}$, $1b^{1.1.1}$, etc. form the apical rosette; and the larger daughter cells, $1a^{1.1.2}$, $1b^{1.1.2}$, etc. form the stem cells of the cross (Plate III, Figs. *P* and *Q*). The rosette cells lie in a depression between the cross cells. The cross cells also become rounded upward and there is a shifting of the $1a^{1.2}$, $1b^{1.2}$, etc. cells to a position between the outer ends of the cross cells. These cells, the $1a^{1.2}$, etc. may then be called the intergirdle cells. The terms "apical rosette," "cross cells," and "intergirdle cells" are used because they have the same origin and relative position as similar cells in *Thalassema*, their ultimate fate not being as yet known in *Urechis*.

The relatively smaller size and the position of the rosette cells in a depression is in contrast with the larger size and more superficial position of the cells in *Thalassema*. In this respect the apical rosette of *Urechis* resembles that of *Podarke*.

PLATE III

FIG. *M*. A lateral view of the 24-cell stage as the cells divide to form the 32-cell stage.

FIG. *N*. A lateral view of the 32-cell stage as the macromeres divide to form the 36-cell stage.

FIG. *O*. A lateral view of the 36-cell stage, showing the characteristic "mushroom" shape of the egg.

FIG. *P*. A polar view of the 36-cell stage showing the division of the micromeres to produce the 40-cell stage.

FIG. *Q*. A polar view of the 40-cell stage showing the apical rosette.

FIG. *R*. A lateral view of the 40-cell stage as the cells divide to form the 48-cell stage.

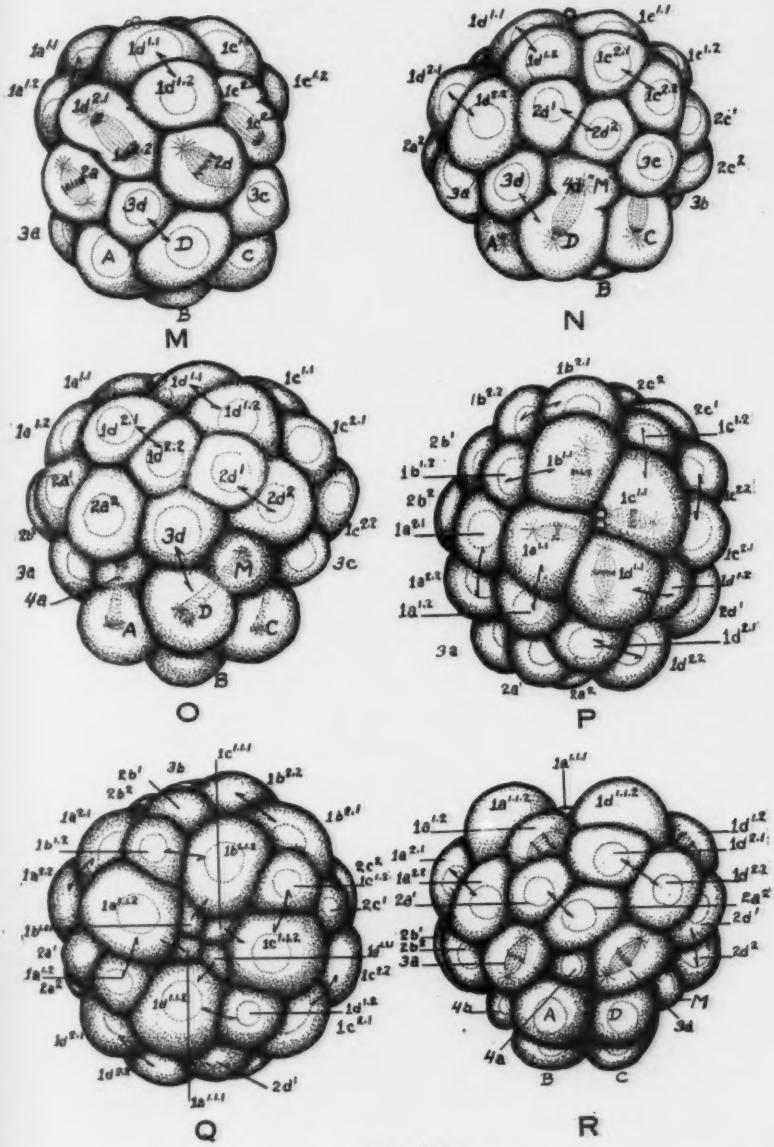


PLATE III

The forty-eight-cell stage.—This stage is formed by an almost simultaneous, leiotropic division of the third quartet cells and the intergirdle cells, the division of the intergirdle cells generally preceding that of the third quartet (Plate III, Fig. *R*). The division of the intergirdle cells is somewhat unequal, the upper cells, lying between the cross cells, being a little larger. The division of the third quartet is also unequal, the upper cells in this case being slightly smaller and rather deeply imbedded between the surrounding prototrochal and second quartet cells.

The fifty-six-cell stage.—This stage is formed by simultaneous, leiotropic, and equal divisions of the prototrochal cells, $1a^{2\cdot1}$, $1a^{2\cdot2}$, etc. (Plate IV, Fig. *S*). The order of these last two cleavages is different in *Thalassema* and *Urechis*. In *Thalassema* there is a division of the third quartet followed by an almost simultaneous division of the intergirdle and prototrochal cells. In *Urechis* the first division takes place simultaneously in the intergirdle and third quartet cells and the second is in the eight prototrochal cells only.

The sixty-four-cell stage.—This stage is formed by a division of the four cross cells, $1a^{1\cdot1\cdot2}$, etc., and the lower moiety of the second quartet cells, $2a^2$, etc. (Plate IV, Fig. *T*). These divisions are important as they mark the end of the purely radial cleavages and initiate the bilateral and morphogenic cleavages. The divisions follow exactly those in *Thalassema* and the symmetry is probably the same. In two adjacent cells of the cross, $1c^{1\cdot1\cdot2}$ and $1d^{1\cdot1\cdot2}$, a meridional, equal division occurs which is shortly followed by an unequal, leiotropic division of the other two cross cells, $1a^{1\cdot1\cdot2}$ and $1b^{1\cdot1\cdot2}$. This later division is almost meridional, the axes of the spindles being inclined only slightly to the left. The smaller cells are budded off toward the center and lie against the rosette cells (Plate IV, Fig. *U*). Bilaterality is now clearly established. However, the relation between the plane of the first cleavage and this plane of symmetry; and the relation between this plane of symmetry and that of the larva has not been determined at this time.

PLATE IV

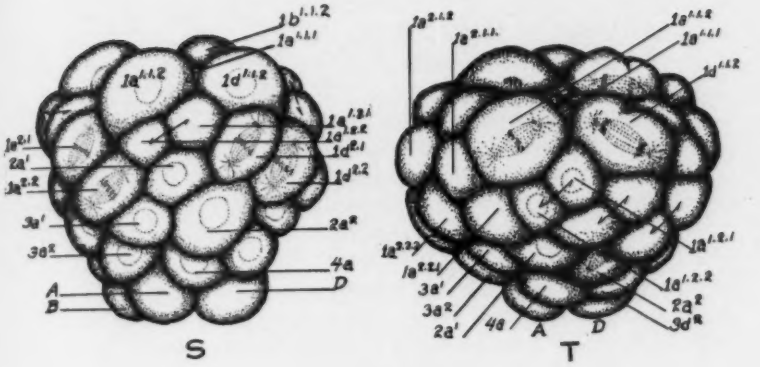
FIG. *S*. A lateral view of the 48-cell stage as the cells divide to form the 56-cell stage.

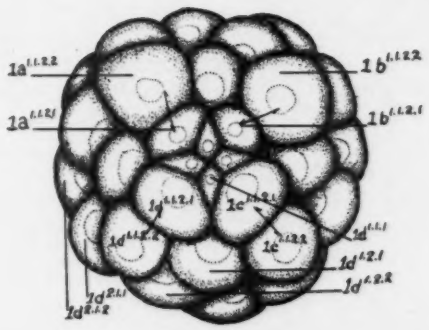
FIG. *T*. A lateral view of the 56-cell stage as the cells divide to form the 64-cell stage.

FIG. *U*. A polar view of the 64-cell stage showing the bilateral symmetry.

FIG. *V*. The right side of the 64-cell stage showing the $2c^{2\cdot2}$ cell as being larger than the $2c^{2\cdot1}$ cell. This is also the case in the division of the $2a^2$ and $2b^2$ cells.

FIG. *W*. A posterior view of the 64-cell stage showing the $2d^{2\cdot2}$ cell as being smaller than the $2d^{2\cdot1}$ cell. This marks the posterior side of the larva.





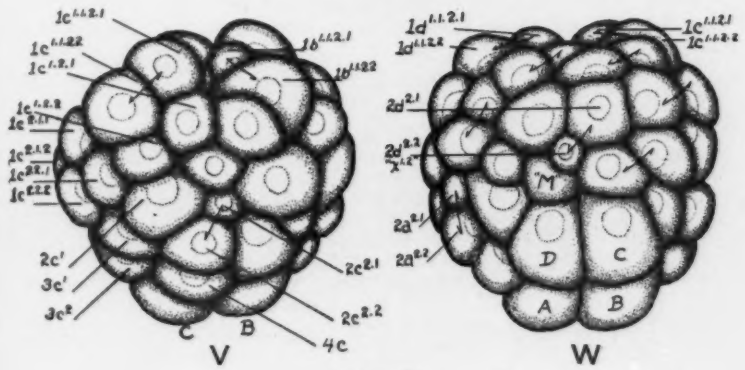


PLATE IV

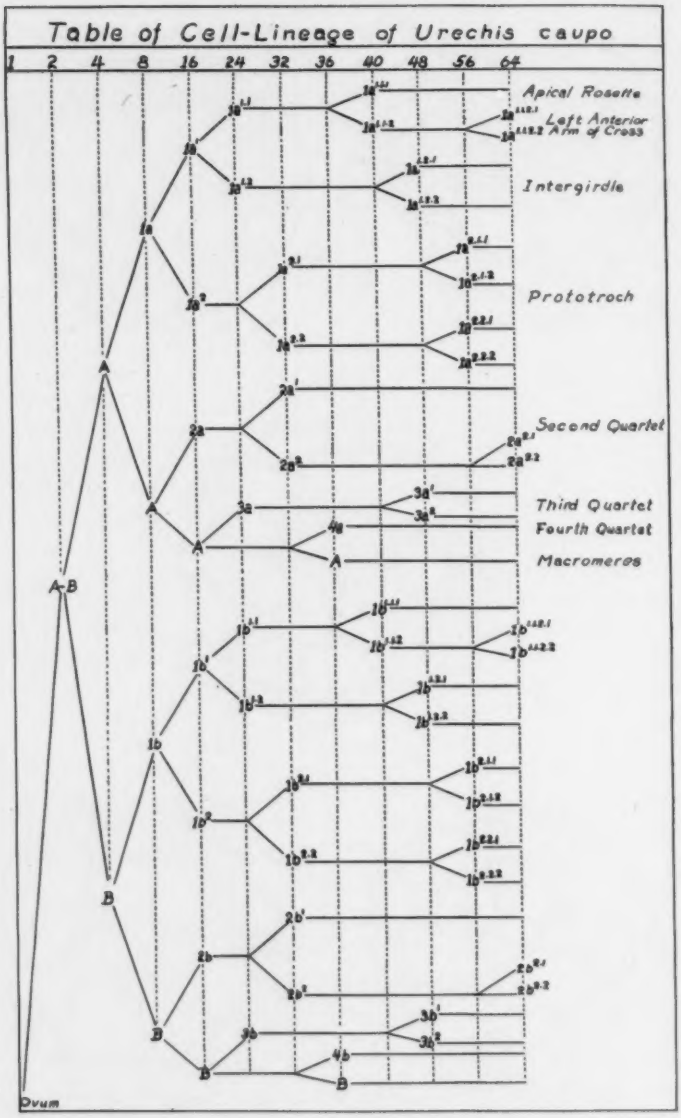


PLATE V

A table which shows the order of the first eleven divisions of the egg of *Urechis*. As the cleavages in all four quadrants are the same at this stage, only the anterior, *A* and *B*, quadrants are shown.

It has been assumed that the first, equal, meridional divisions of the cross cells take place in the posterior, *C* and *D*, quadrants as they do in *Thalassema*. This is further confirmed by the mode of division of the second quartet cells. In the $2a^2$, $2b^2$, and $2c^2$ cells the unequal, leiotropic divisions have the smaller cells budding off upwardly to become deeply imbedded between the surrounding cells. The $2d^2$ cell also divides unequally but with the smaller cell downward, overlying the 4*d* or *M* cell. This cell has been designated by Treadwell for *Podarke*, and Torrey for *Thalassema* as the $x^{1\cdot2}$ cell.

"Treadwell ('97 and '01) in his discussion of cell-homologies calls attention to the fact that this cell has the same origin in all the forms of annelids and molluscs that have been studied." (Quoted from Torrey.) The identity of the four quadrants and the plane of symmetry is thus established before the division of the *M* cell.

The sixteen prototrochal cells have a rather superficial position on the surface of the egg and do not extend inward to the segmentation cavity. The fertilization membrane persists around the entire egg but at the animal pole has a depression that brings it in contact with the apical rosette cells.

Development to the 64-cell stage takes five and one-half to six hours at 18° to 20° C. Cilia develop on the prototroch rather rapidly between six and a quarter and six and one-half hours and almost immediately begin to beat, causing the egg to rotate. The longer apical cilia develop later.

Conclusion

Except for the shape of the egg and differences in the relative sizes of some of the cells the egg of *Urechis* at the 64-cell stage is identical with that of *Thalassema* and similar to the eggs of other annelids with equal cleavages.

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POLYVITELLINE EGGS AND DOUBLE MONSTERS IN THE POND SNAIL LYMNÆA COLUMELLA SAY

CHARLES P. WINSOR AND AGNES A. WINSOR

DEPARTMENT OF BIOLOGY, SCHOOL OF HYGIENE AND PUBLIC HEALTH, JOHNS HOPKINS UNIVERSITY

The occasional occurrence of gastropod eggs containing more than one embryo is more or less familiar to workers with these forms. Such embryos are normally separate; but occasionally conjoined twins are found, and more rarely triplets, and instances of as many as five conjoined embryos have been reported. The literature on this subject has been well reviewed by Pelseneer (1920) and Crabb (1931), so that no extended historical discussion seems necessary. Our purpose is merely to present certain material which seems to us useful in supplementing and perhaps in modifying the conclusions of other workers.

The material here presented consists of the total egg production throughout life of a population of (initially) 400 snails of the species *Lymnæa columella* Say. The wild parents of these snails were collected in two ponds in the vicinity of Baltimore, designated here as the Falls Road pond and the Boyce Avenue pond. In addition to these wild ancestors of known origin, two snails isolated from laboratory aquaria furnished eggs for this experiment; nothing is known concerning their origin. These animals were isolated in the laboratory in finger bowls with about 150 ml. of spring water, fed with leaf lettuce, and their eggs collected daily. The eggs so obtained were allowed to develop for about a week, at which time healthy-appearing clutches were selected for the experimental population. These eggs were removed from the capsule and placed in finger bowls with spring water. Each clutch of eggs provided twenty eggs, which were distributed among six finger bowls, one at density ten and five at density two per bowl. Leaf lettuce was used for food. Records of egg production were kept for each dish throughout the lives of the animals. Early in the experiment appreciable numbers of polyvitelline eggs appeared and were regularly entered in the records. Occasional double monsters were found; such eggs were saved and their development followed until the death of the embryos.

Table I summarizes the results obtained.

The facts regarding the production of polyvitelline eggs are summarized in Fig. 1. This chart shows the percentage of polyvitelline

eggs laid in each finger bowl in the experiment. The parentage of the snails in each finger bowl is also shown, together with the geographical origin of the parents.

It is clear from an examination of the chart that there are very marked differences in the proportions of polyvitelline eggs occurring in different finger bowls; and that there is clear indication that the offspring of wild parents from the Falls Road pond, especially those

TABLE I

Production of Polyvitelline Eggs by Offspring of Different Wild Parents

Wild ancestor	No. of dishes	No. of snails	Total eggs	Total polyvitelline eggs	Percentage polyvitelline eggs	Eggs having given number of vitelli							
						2	3	4	5	6	7	8	9 and over
1	5	8	3,570	58	1.62	47	9	2	—	—	—	—	—
2	17	42	34,199	1,453	4.25	1,079	272	63	15	4	8	3	9
4	16	35	37,761	1,631	4.32	1,166	324	89	20	13	6	5	8
6	2	2	3,031	146	4.82	103	36	4	1	1	1	—	—
8	12	22	9,857	144	1.46	110	23	4	3	2	1	—	1
Totals 1–8 Falls Road	52	109	88,418	3,432	3.88	2,505	664	162	39	20	16	8	18
12	12	23	14,508	96	0.66	85	8	2	—	1	—	—	—
15	6	14	9,647	81	0.84	65	12	—	1	—	—	3	—
16	12	29	17,672	244	1.38	220	17	1	3	1	1	—	1
17	5	10	7,359	106	1.44	83	14	4	1	3	—	—	1
Totals 12–17 Boyce Ave.	35	76	49,186	527	1.07	453	51	7	5	5	1	3	2
A2	7	10	13,411	227	1.69	209	8	3	3	1	—	—	3
A3	6	11	13,369	73	0.55	63	3	7	—	—	—	—	—

Notes: Column 1 shows the wild ancestor. Numbers 1 to 8 inclusive were taken from the Falls Road pond; numbers 12 to 17 from the Boyce Avenue pond. Numbers A2 and A3 were isolated from a laboratory aquarium culture; their wild origin is unknown.

from Nos. 2 and 4, laid a considerably higher proportion of polyvitelline eggs than those from the Boyce Avenue pond. There seems to be reason for supposing that these differences are the expression of some inherited character.

This conclusion differs from that of Crabb, who holds that there is no indication that the production of polyvitelline eggs is influenced by hereditary factors. The data given by the Crabbs (1927) seem to us, however, consistent with the notion that inheritance does play a

part in the phenomenon. Neither their data nor ours, however, are adequate to suggest any Mendelian mechanism.

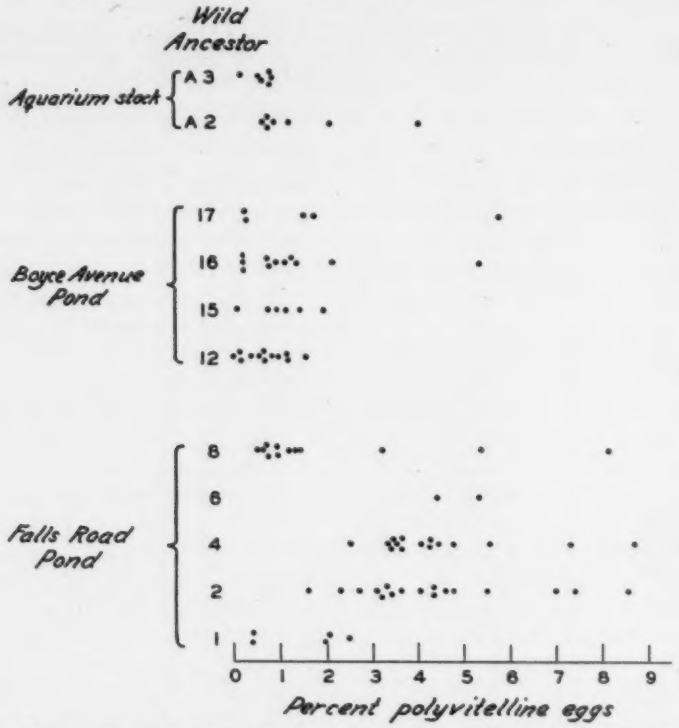

FIG. 1. Chart showing proportion of polyvitelline eggs in different finger bowls, segregated by ancestry.

A paper by Tur (1910) (not cited by Crabb) is of interest in this connection. He found, in *Philine aperta*, that there were distinct local races, some of which produced polyvitelline eggs with some frequency and regularity, while animals from other localities rarely or never laid such eggs.

EXPLANATION OF PLATE

FIGS. 1–3. Lateral fusion with separate shells. Three views. Parent No. 27, grandparent No. 2.

FIGS. 4–5. Dorsal fusion with separate shells. Two views. Parent No. 7, grandparent No. 4.

FIG. 6. Visceral masses fused, single shell. Parent No. 12, grandparent No. 4.

FIGS. 7–8. Triple monster, shell fused. Parent No. 26, grandparent No. 2.

FIG. 9. Dorsal fusion, single shell. Parent No. 11, grandparent No. 4.

1

2

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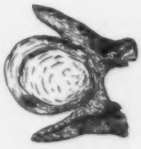

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Double Monsters

Double monsters in considerable numbers were observed in our material. Drawings of typical specimens (made for us by the laboratory artist, Mr. Arthur Johansen) are shown in Plate I. As regards the anatomical details of these monsters, we can add nothing of importance to Pelseneer's account. So far as our observations went, they agreed with his.

It will be noted that the wild ancestors giving rise to these double monsters came almost entirely from the Falls Road pond, as would be expected in view of their apparently certain origin through fusion of embryos. Actually, of 32 double and triple monsters, 28 (of which one was triple) had a Falls Road origin; one was derived from Boyce Avenue stock; and three (one triple) from laboratory stock of unknown origin.

Summary

Data are presented regarding the production of polyvitelline eggs and double monsters through the entire life of a group of *Lymnæa columella*. It is held that the data show that hereditary factors are concerned.

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Detailed bibliographies will be found in Crabb (1931) and Pelseneer (1920).

STUDIES ON AMPHIBIAN METAMORPHOSIS

X. HYDROGEN-ION CONCENTRATION OF THE BLOOD OF ANURAN LARVÆ DURING INVOLUTION[1]

O. M. HELFF

DEPARTMENT OF BIOLOGY, UNIVERSITY COLLEGE, NEW YORK UNIVERSITY

INTRODUCTION

The metamorphosis of anuran larvæ, aside from the striking embryological and growth phenomena exhibited, presents perhaps an even more interesting series of degenerative changes, involving cellular autolysis and various histolytic effects, which in some cases results in the total atrophy of an organ. The range of tissues so affected and the degree to which they degenerate is subject to considerable variation, however. Thus we find the tail, the gills, and the areas of opercular integument through which the fore-limbs emerge undergoing complete atrophy resulting in the total resorption of the tissues concerned, with the exception of the gills which are represented in the adult by small spherical bodies, the "Kiemenreste," whose function, if any, is unknown. On the other hand, organs such as the intestines, stomach, and pancreas which undergo a reorganization in the development of the adult condition, are subject to a lesser degree of atrophy, although the amount of cellular histolysis is very great. Contrasted to the above two categories of degenerating tissues, we find those in which relatively little degeneration occurs as in the tissues of the jaws during the shedding of larval teeth, the early degenerative changes of integument in the formation of the tympanic membranes, and the general atrophy of the musculature of the back, the latter being subject to approximately a 38 per cent reduction in volume during metamorphosis. Although relatively little, if any, information is available regarding the histological changes operating in the case of the jaw tissues, pancreas, and gills, more definite data can be found in relation to the other organs and tissues as cited above. For such information the reader is referred to the papers of Barfurth (1887), Bataillon (1891), Bradley (1922), Clausen (1930), Helff (1926) (1928) (1931*a*), Helff and Clausen (1929), Kuntz (1922) (1924), Lindeman (1929*a*), Mercier (1906), Morse (1918), and Van Der Jagt (1929).

[1] The expenses entailed in this investigation have been partially met by a grant of money from the Bache Fund of the National Academy of Sciences.

Although the endocrine glands, notably the thyroid and anterior pituitary, are generally conceded to furnish the initial stimuli for amphibian metamorphosis, relatively little information is available in respect to their mode of action. It seems quite certain, however, that certain developments and degenerative processes are not brought about by the endocrine hormones acting directly on the tissue concerned through the medium of the blood stream. Thus the dedifferentiation of integument to form the tympanic membranes is due to contact of the integument with the developing annular tympanic cartilage and columella (Helff, 1928 and 1931), while the total histolysis of opercular integument as it occurs in the formation of the fore-limb perforations is the result of integumentary contact with the atrophying tissue of the gills (Helff, 1926). On the other hand, the development and degeneration of tissues following transplantation to foreign regions strongly suggests the operation of metamorphic influences brought into play through the blood supply to the transplant. The development of the tongue (Helff, 1929), the columella (Helff, 1931*a*), the dermal plicæ (Helff, 1931*b*), the fore-limbs (Helff, 1926), the hind-limbs (Schubert, 1926), the adult pigment pattern (Lindeman, 1929*a*) and the nictitating membrane (Lindeman, 1929*b*) are all cases in point in which the developmental stimulus is through the blood stream. The degenerative changes which have been shown to occur normally upon transplantation to foreign regions include the histolysis of tail integument (Lindeman, 1929*a*, and Clausen, 1930), and the histolysis of tail muscle (Helff and Clausen, 1929). Indications also point to the probability that future work will add the atrophy of the gills, intestines, and pancreas to this latter group.

While it is possible that all of the developmental and atrophic changes which occur during amphibian metamorphosis may be due to hormonic influences in the blood stream, it seems rather improbable that such will ultimately prove to be the case in relation to the partial or total histolysis of larval organs. Due to the considerable mass of work that has been done on various tissues in widely divergent animal groups in relation to the biochemical factors associated with and favorable to the autolysis of living tissues "in vivo" and "in vitro," one cannot escape the implication that the histolysis of larval organs and tissues in the amphibian is due to a more immediate and plausible causative factor which is the result, primarily, of course, of the metamorphic hormones. An obvious factor and one that has been shown to be closely correlated with the autolysis and atrophy of a wide variety of tissues in other classes of animals, is that of the hydrogen-ion concentration of the medium in which the autolysis occurs. Briefly,

it may be stated that in general the increasing of the hydrogen-ion concentration of the medium above a certain level either tends to activate the proteolytic enzymes concerned in autolysis or else brings about changes in the tissue proteins themselves which render them digestible by the omnipresent enzymes.

The possibility of a lowering in the pH of the blood during amphibian metamorphosis has been suggested by many writers as a likely explanation for many of the histolytic processes which occur. Thus Barfurth (1887), Bataillon (1891), Mercier (1906), Morse (1918), and Bradley (1922) all suggest the probability that the tissues of the larval tail undergo autolysis in response to a localized increase in hydrogen-ion concentration, the result of a partial or total occlusion of the dorsal aorta resulting from pressure exerted by the rapidly growing urostyle. Although their assumption of a lowered pH as the immediate causative factor may have been correct, the importance of the developing urostyle in this connection was found to be negligible as shown by subsequent work. In this connection it may be stated that the writer (1930) was able to show that the process of tail resorption was not inhibited in any way in the complete absence of the urostyle, the latter having been extirpated while still in the anlage stage prior to the onset of larval involution. It was also shown by Lindeman (1929*a*) that tail integument previously transplanted to the back underwent normal histolysis during metamorphosis at the same time the integument on the tail autolyzed. The same result was obtained by Helff and Clausen (1929) working with tail muscle.

A consideration of the foregoing brief survey regarding the problem of autolysis during larval involution clearly suggests that the question of hydrogen-ion concentration of the blood before, during, and after metamorphosis becomes a problem of considerable interest and importance. As far as the writer is aware, there is no previously published work relating to the hydrogen-ion concentration of larval blood either before or during metamorphosis. Numerous tests have been made on the blood of the adult amphibian, however, in relation to various environmental and experimental factors. In this relation the reader is referred to the papers of Rohde (1920), Hertwig (1927), Kamm (1930), et al. The present paper, therefore, relates the results of hydrogen-ion tests as made on the blood of non-metamorphosing larvæ, larvæ in various stages of involution, and young frogs shortly after the completion of metamorphosis.

Materials and Methods

The initial stock used for all hydrogen-ion tests were large second-year *Rana clamitans* larvæ obtained at regular intervals from a common

source near Cincinnati, Ohio, during the winter and spring months of 1930 and 1931. The selection of *Rana clamitans* larvæ was based on two points both favorable to the work at hand; first, in that the large size of the tadpoles insured a sufficient quantity of blood for testing purposes and second, that due to the neotenous nature of the species and the fact that they were second-year larvæ, normal metamorphosis could be induced when placed in water at room temperatures during the spring months especially. Shipments of larvæ, when received, were first placed in a large tank in order to acclimate them to the new water medium and also to allow the less hardy to die out. Under laboratory conditions larvæ also frequently tend to develop edema and red-leg, cases of which were always eliminated during this preliminary quarantine period. In testing the normal or Stage 1 larvæ, the tadpoles were first isolated in individual bowls of water for a period of at least three days prior to the hydrogen-ion testing. In obtaining the various states of metamorphosis tested, Stage 1 larvæ were likewise isolated in individual bowls of water and allowed to metamorphose normally. In both cases no food was given to the animals. The time required at room temperatures for complete metamorphosis to occur following the onset of involution varied from four to seven weeks.

The method employed in obtaining samples of blood for testing purposes was as follows: The larvæ or newly metamorphosed frogs were first fastened, dorsal side down, to a moistened pad of cloth by means of rubber bands. It may be said in this connection that the elimination of the use of an anæsthetic in the securing of the blood samples was thought to be advisable in that the pH of the blood might be altered by such a procedure. Two incisions were now made through the integument of the anterior ventral surface; one running from side to side just anterior to the level of the heart, and the other along the mid-ventral line from the middle of the first incision anterior to the rim of the lower jaw. The two flaps of integument were now pulled aside, laterally, so as to expose the general region anterior to the heart. In the case of larvæ this exposes the pericardial sac, while in the young frog it is first necessary to resect certain underlying muscular layers before the pericardium is brought to view. Following the exposure of the pericardial sac, the latter is cut at a point to expose the truncus arteriosus. In so doing, care must be exercised not to cut too far posterior or else premature bleeding may occur due to injury of the anterior abdominal vein. The cutting of the pericardial sac liberates, especially in normal larvæ, a considerable amount of lymph which quickly flows out, however. The lymph is now carefully removed from the cavity so prepared immediately anterior to the heart.

The truncus arteriosus is now cut through using a small Noyes' iris scissors and blood quickly flows out filling the prepared cavity anterior. The blood is quickly drawn up into a pipette which has been previously rinsed with a 2 per cent solution of sodium oxalate. The small amount of oxalate solution adhering to the walls of the pipette is sufficient to prevent the clotting of the blood. The blood is now immediately transferred to a small vial and the hydrogen-ion concentration test quickly made.

The determination of hydrogen-ion concentration was made by means of the quinhydrone method. In this regard it seems advisable to mention some of the more important technical points found advantageous in securing greater accuracy with this method. Care must be exercised in the cleaning of the blood vials. The routine followed was to first thoroughly wash the vials and then rinse several times with hydrochloric acid followed by alcohol and distilled water. The vials were then allowed to dry thoroughly before using. Due to the small quantity of blood tested, very little quinhydrone had to be added. Solution of the latter in the blood was facilitated by gently tapping the vial for a few seconds. The platinum leaf electrode was next inserted to the bottom of the vial. By bending the platinum leaf at right angles to the supporting capillary glass tube, the former could be made to lie flat on the bottom of the vial and thus insure contact with the blood on both surfaces. Care should also be taken to carefully cleanse the test electrode between any two consecutive tests by rinsing thoroughly in hydrochloric acid, alcohol, and distilled water. The electrode should be dried carefully before using again. The agar bridges employed were of the usual type except that quite small tubing was necessary due to the small diameter of the blood vials. Care should be taken to cleanse the end of the agar bridge which has been immersed in the blood of the test vial, between individual tests. The quicker the e.m.f. is determined the better, while use of a room with little variation in temperature also makes for greater accuracy, especially where comparative results are desired. Finally, it is a good practice to standardize the set at frequent intervals by checking against a standard solution of 1/20 molar potassium acid phthalate which should give a pH of 3.98 at 25° C. In the present work such a standardization test was made between every two consecutive blood tests.

Results

Blood pH of Normal, Non-metamorphosing Larvæ

The larvæ used for the Stage 1 tests were normal tadpoles in all respects which would not have begun metamorphosis for at least a

TABLE I

Measurements of pH of Blood of Rana clamitans at Different Stages in Metamorphosis

Stage 1 Normal larvæ			Stage 2 First metamorphic change			Stage 3 One fore-leg emerged			Stage 4 Complete metamorphosis		Stage 5 3 to 4 weeks after Stage 4	
Weight	Length*	pH	Weight	Length*	pH	Weight	Length*	pH	Weight	pH	Weight	pH
gms.	*mm.*		*gms.*	*mm.*		*ms.*	*mm.*		*gms.*		*gms.*	
8.0	15 × 105	7.54	5.3	18 × 95	7.41	2.7	23 × 83	7.18	2.4	7.29	1.5	7.24
7.7	8 × 103	7.54	6.4	20 × 97	7.36	4.7	32 × 92	7.19	2.7	7.18	2.2	7.19
7.4	14 × 100	7.43	5.6	17 × 88	7.38	3.6	31 × 85	7.35	2.0	7.16	1.7	7.14
8.1	13 × 105	7.52	5.8	19 × 95	7.45	4.3	37 × 95	7.28	2.5	7.19	1.9	7.15
7.0	15 × 93	7.49	4.9	21 × 95	7.47	3.3	28 × 85	7.20	1.8	7.23	1.7	7.18
8.2	10 × 105	7.54	5.2	22 × 97	7.40	4.0	27 × 90	7.28	2.1	7.10	2.4	7.22
8.6	12 × 105	7.54	4.7	19 × 98	7.44	4.0	33 × 88	7.32	2.3	7.24	2.3	7.18
7.4	8 × 102	7.54	4.8	19 × 100	7.34	3.4	30 × 82	7.23	2.5	7.17	1.8	7.19
7.6	9 × 102	7.52	4.6	16 × 90	7.39	5.0	33 × 94	7.28	2.5	7.13	2.4	7.11
7.4	10 × 92	7.45	5.2	18 × 96	7.42	5.1	36 × 97	7.34	3.0	7.26	2.1	7.26
7.2	13 × 98	7.47	5.0	19 × 97	7.40	4.7	32 × 85	7.31	2.7	7.19	1.9	7.19
8.2	17 × 100	7.49	4.7	20 × 99	7.37	3.8	24 × 85	7.24	2.3	7.20	2.0	7.14
7.2	14 × 101	7.53	4.9	19 × 96	7.44	3.9	26 × 91	7.21	2.4	7.16	2.1	7.16
6.7	8 × 98	7.43	5.1	21 × 97	7.37	4.1	25 × 87	7.26	2.0	7.18	2.0	7.20
6.7	8 × 102	7.50	4.8	21 × 99	7.33	4.5	34 × 92	7.28	2.6	7.25	2.5	7.24
7.2	11 × 98	7.54	5.4	18 × 97	7.41	3.7	30 × 90	7.33	2.2	7.22	2.1	7.25
7.6	10 × 101	7.49	4.6	22 × 100	7.28	3.9	28 × 88	7.25	1.9	7.17	2.0	7.28
8.1	9 × 102	7.52	5.0	21 × 94	7.35	4.4	31 × 91	7.31	2.5	7.26	1.8	7.15
7.4	13 × 94	7.47	5.1	17 × 95	7.44	3.6	30 × 93	7.27	2.3	7.24	1.4	7.23
7.7	12 × 100	7.52	4.7	19 × 96	7.38	4.5	28 × 91	7.24	2.4	7.18	2.1	7.18
7.3	15 × 102	7.53	4.9	19 × 99	7.36	3.8	25 × 86	7.28	1.9	7.23	1.7	7.12
7.6	10 × 101	7.48	5.5	18 × 93	7.45	3.7	32 × 96	7.30	2.7	7.27	1.9	7.15
8.1	12 × 104	7.50	5.2	20 × 94	7.44	3.9	23 × 84	7.24	2.3	7.17	2.0	7.11
7.3	9 × 99	7.53	4.4	21 × 97	7.31	4.1	27 × 86	7.33	2.6	7.20	1.8	7.12
7.8	11 × 103	7.49	4.8	19 × 95	7.33	4.3	28 × 92	7.23	2.1	7.18	1.5	7.14

* Hind-limb length × total length.

month. Their hind-limb lengths varied from 8 to 17 mm., while in total length the variation was between 92 and 105 mm. The weight of such individuals varied from 6.7 to 8.6 grams.

Of the various stages tested, Stage 1 larvæ were most favorable in respect to the quantity of blood obtainable. Although some variation was experienced in this regard, a sufficient amount was always present. On the average about one-tenth of a cubic centimeter could be secured per animal. Columns 1, 2, and 3 of Table I present the essential data for this group of tests. It will be seen that the range of pH was not great, being from a minimum of 7.43 to a maximum of 7.54 and representing a variation, therefore, of but 0.11 of a pH unit. The average pH of the twenty-five larvæ tested proved to be 7.50 ± .00459, while the standard deviation amounted to .034 ± .0032 (see Table II). We can thus consider a pH of 7.50 as typical for the blood of normal non-metamorphosing larvæ of the particular species being tested.

TABLE II

Means, Standard Deviations, and Probable Errors

Stage	Mean	Standard deviation
1	7.50 ± .00459	.034 ± .0032
2	7.39 ± .00650	.048 ± .0046
3	7.27 ± .00634	.047 ± .0045
4	7.20 ± .00607	.045 ± .0043
5	7.18 ± .00648	.048 ± .0046

Blood pH During Metamorphosis

The various stages of metamorphosis tested were obtained by allowing natural metamorphosis to take place. The isolation of Stage 1 larvæ in individual bowls of water at room temperature acted as a stimulus to transformation with the result that the onset of metamorphosis usually began in about three weeks following their transfer from the stock tanks of cold running water. All cases of edema and red-leg which occurred during metamorphosis were discarded. During the early stages of involution the mortality was relatively low. The acquiring of completely metamorphosed larvæ, however, involved a mortality of over 80 per cent, necessitating the isolation of over one hundred and twenty-five Stage 1 larvæ in the ultimate securing of twenty-five Stage 4 animals. To obtain twenty-five Stage 5 animals, it was necessary to isolate a still larger number of Stage 1 larvæ, since the mortality was even greater in that about one-fourth of the Stage 4 animals died within three to four weeks following the attainment of

that stage. In all, some four hundred and thirty Stage 1 larvæ were isolated in securing twenty-five cases each of Stages 1, 2, 3, 4, and 5.

Stage 2.—Stage 2 larvæ were tadpoles which had attained early metamorphic changes characterized by a definite acceleration in fore- and hind-limb growth, the loss of the full-bellied appearance typical of Stage 1 larvæ, a slight anterior-posterior atrophy of the tail with pronounced histolysis of the dorsal and ventral finny portions, and a general darkening of integumentary pigmentation. The hind-limbs varied between 17 and 22 mm. in length, and total length between 88 and 100 mm. The weight varied between 4.6 and 6.4 grams.

The fourth, fifth, and sixth columns of Table I present the essential data for Stage 2 tests. It will be noted that the range in pH was somewhat greater than in the case of the Stage 1 tests, being from 7.28 to 7.47 or a variation of 0.19 of a pH unit. The average pH of the twenty-five larvæ tested amounted to $7.39 \pm .00650$, the standard deviation being $.048 \pm .0046$. The results are clear, therefore, in that a definite change in pH from 7.50 to 7.39, representing a drop of 0.11 of a pH unit, occurs during this early stage of metamorphosis.

Stage 3.—Stage 3 larvæ represented a more pronounced stage of larval involution. At this stage the left fore-limb had just emerged, while the hind-limbs were considerably increased in length. The tail had undergone still further atrophy and the intestines were rapidly being transformed into the adult type. The hind-limb lengths varied between 23 and 37 mm., and total length between 82 and 97 mm. The drop in weight was especially pronounced, the larvæ varying between 2.7 and 5.1 grams.

The ninth column of Table I presents the hydrogen-ion concentrations determined for Stage 3 larvæ. The range in pH is from 7.18 to 7.35 or a variation of 0.17 of a pH unit. The average pH of the twenty-five tests amounts to $7.27 \pm .00634$ with a standard deviation of $.047 \pm .0045$. A still further drop of 0.12 of a pH unit on the average has apparently occurred, therefore, due to the more advanced stage of metamorphosis attained.

Stage 4.—Stage 4 individuals were characterized by practically complete metamorphosis. The animals were fully metamorphosed except for the persistence of a small 5–10 mm. tail stump and the lack of tympanic membrane development. Pigmentation, jaw development, and sitting posture were typically frog-like. Body and hind-limb measurements were not made, but the weight was found to vary between 1.8 and 3.0 grams.

The individual pH determinations of Stage 4 animals are listed in column 11 of Table I. The range is from 7.10 to 7.29, or a variation

of 0.19 of a pH unit. The average pH amounted to 7.20 ± .00607 and the standard deviation was .045 ± .0043 (Table II). The average pH therefore indicates a still further drop in concentration amounting, on the average, to 0.07 of a pH unit.

Blood pH Following Metamorphosis

Newly-metamorphosed young frogs were selected for the final group to be tested in order to determine whether or not the drop in pH, as it occurs during metamorphosis, is a transient phenomenon or is an actual adjustment of the blood to a hydrogen-ion concentration typical of the adult of the species concerned. The weights of Stage 5 animals varied between 1.4 and 2.5 grams. The average weight was somewhat less than that of Stage 4 animals, due no doubt to the fact that they had been without food for from 3 to 4 weeks.

Column 13 of Table I lists the individual pH determinations made. The range is from 7.11 to 7.28 or a variation of 0.17 of a pH unit. The average pH of the entire group amounts to 7.18 ± .00648 with a standard deviation of .048 ± .0046 (see Table II). The difference between pH 7.20 (the average for Stage 4) and 7.18 (the average for Stage 5) is insignificant and well within the probable and experimental errors. Hence it seems probable that this pH represents, approximately, the hydrogen-ion concentration of the adult frog's blood for the species in question. This point, however, is a debatable one and will be further taken up in the next section of this paper.

Discussion

The variation in blood pH between Stage 1 larvæ, although not excessive, calls for some explanation. The greatest difference between any two of the twenty-five larvæ tested amounted to 0.11 of a pH unit. This variation can be partially accounted for by the limitations of the quinhydrone method which admits of an accuracy to within only 0.04 of a pH unit. It seems quite probable, however, that a definite amount of variation actually exists between various individuals of the same developmental stage in that even greater differences have been recorded in the adult frog. Thus Kamm (1930), working with fully-grown *Rana esculenta* and *Rana temporaria*, records blood readings ranging from pH 7.36 to pH 7.61 in cases of starved animals. On the same species Hertwig (1927) obtained variations ranging from pH 7.36 to pH 7.59, while Rohde (1920) recorded a minimum pH of 6.32 and a maximum pH of 7.13. Kamm has pointed out, however, that the extreme variations as recorded by Rohde were probably due to certain technical errors. The wide variations as recorded by Kamm and

Hertwig, however, give evidence of the differences in hydrogen-ion concentration of the blood that exist between individual adult frogs of the same size and developmental condition. Apparently the blood of the amphibian is very poorly buffered as compared with the condition found in the mammals. This point is strongly emphasized by Kamm's (1930) work in which the pH of frog's blood was quickly lowered by feeding with boric acid and as quickly raised by sodium carbonate feeding. It seems quite likely, therefore, that the blood pH differences as recorded in the present paper for normal larvæ represent actual differences between individuals, the result of a relatively poor controlling mechanism for maintaining a constant hydrogen-ion concentration of the blood stream. In this connection it should be stated, however, that the comparatively greater extremes recorded between individuals in Stages 2, 3, 4, and 5 were no doubt partially due to the difficulty of selecting twenty-five animals of exactly the same developmental stage.

TABLE III

Comparison of Mean Differences and their Probable Errors

Stages compared	Differences between means	Probable error of the difference	Difference / Probable error of the difference	Average percentage decrease between means
				per cent
1 and 2.....	.11	.007957	13.8	1.5
2 and 3.....	.12	.009080	13.2	1.6
3 and 4.....	.07	.008777	8.0	1.0
4 and 5.....	.02	.008879	2.3	.3

The results of the present paper clearly indicate that an actual lowering of blood pH occurs during metamorphosis. The drop in pH would appear to be a gradual one, although somewhat more pronounced in the earlier stages of involution. That the various differences recorded between the means of Stages 1, 2, 3, and 4 are significant ones is shown by an analysis of such differences in relation to their respective probable errors (see Table III). Thus the difference between the means of Stages 1 and 2 or 0.11 of a pH unit is 13.8 times its probable error while the differences between Stages 2 and 3, and 3 and 4, or 0.12 and 0.07 of a pH unit, respectively, are 13.2 and 8.0 times their probable errors. Hence the differences as recorded are significant and represent an actual increase in hydrogen-ion concentration.

The difference between the means of Stages 4 and 5 (see Table III), or 0.02 of a pH unit, being only 2.3 times its probable error indicates

that no significant change in hydrogen-ion concentration of the blood had occurred within 3 to 4 weeks following the close of the metamorphic period. Whether this represents the condition typical of young frogs newly metamorphosed in a natural environment is a questionable point. The experimental animals in the laboratory were not fed and hence were in a starved condition when tested. Young frogs in their natural outdoor environment would be feeding at this stage and increasing their weight instead of losing weight as was typical of the experimental animals (see Stage 4, Table I). In this relation it may be stated that feeding in the fully-grown frog apparently affects blood pH; according to Kamm (1930) who found the pH higher in the case of laboratory-starved animals as compared with freshly caught, well-nourished frogs. It seems quite probable, however, that the hydrogen-ion concentration of the blood may decrease somewhat in *Rana clamitans* as the latter attain their full size during successive years of growth, especially since Kamm (1930) and Hertwig (1927), have recorded concentrations well above pH 7.3 for fully-grown adults of *Rana esculenta* and *Rana temporaria*. However, it is possible that the pH of adult *Rana clamitans* blood is characteristically lower than that of the species tested by Kamm and Hertwig.

The relationship of the increased hydrogen-ion concentration during metamorphosis to the various degenerative changes which occur may be of several types. In the first place, it is evident that the pronounced autolytic processes operating no doubt account for an accumulation of acid metabolites in the blood stream resulting in an increase in hydrogen-ion concentration of the latter. However, it is possible that the first lowering of the pH actually precedes the onset of, and may be causative for, the early autolytic degenerations which occur in that the increased hydrogen-ion concentration either activates the autolytic enzymes or increases their rate of action in the digestion of tissue proteins. Again the tissue proteins may simply be rendered more easily digested. Regarding the above points, it should be noted that the results of the present paper (due to the necessity of killing the larvæ in order to obtain sufficient blood for the quinhydrone test) do not serve to indicate clearly whether or not a drop in pH actually precedes the first degenerative changes. It would be interesting in this connection to study the pH changes of the blood in the same individual larva immediately before and after the first degenerative tissue changes occur. This could be done with a suitable micro-electrode hydrogen-ion apparatus and without killing the larva due to the small amount of blood necessary for the test. Even if such tests should show that pH changes are purely secondary in time

sequence to early degenerative changes, the probability still would remain that the heightened hydrogen-ion concentration of the blood which has been shown to occur by the results of the present paper at a relatively early stage of metamorphosis, are either causative or at least favorable to the later autolytic changes which occur during larval involution. The fact that the various organs and tissues which undergo histolysis do not degenerate all at the same stage of metamorphosis but have a definite time sequence coincident with various degrees of involution, strongly suggests a correlation between a definite blood pH and the onset of autolysis of each organ.

The writer wishes to point out the possibility that carbon dioxide escape may have affected the actual pH readings recorded for the various tests. Such escape no doubt occurred to a certain extent during the collecting and transferring of the blood samples to the electrode vessels, although the general agreement of the results obtained for any one stage of metamorphosis would seem to indicate that if such losses did occur, they were not great. Consequently, although finer micro-electrode methods coupled with suitable carbon dioxide correction factors may ultimately serve to alter the average hydrogen-ion concentrations recorded for the various metamorphic stages, it seems quite unlikely that the approximate difference of 0.3 of a pH unit between normal larval and completely metamorphosed larval blood will be seriously affected. The important point to be derived, therefore, from the present paper is not that the results strive to represent a final determination of the exact average pH typical of each stage of metamorphosis, but rather that a definite drop in pH does occur during larval involution which may be and probably is correlated with various metamorphic structural and physiological changes.

Summary and Conclusions

1. The blood pH of twenty-five normal larvæ of *Rana clamitans* was determined by means of the quinhydrone method. An average pH of 7.50 was obtained.

2. Two intermediate stages of metamorphosis were tested (twenty-five cases of each) showing an average progressive decrease in pH to 7.39 and 7.27, respectively.

3. The average blood pH of twenty-five animals, immediately following the completion of involution, amounted to 7.20. It is concluded that a definite increase in the hydrogen-ion concentration of anuran blood occurs during larval transformation amounting to an approximate drop of 0.3 of a pH unit.

4. The average blood pH of newly-metamorphosed frogs, three to four weeks after the completion of metamorphosis, was determined as approximately 7.18. It is concluded that little, if any, change in blood pH occurs within three to four weeks following the completion of larval involution, although changes may occur during future adult growth.

5. The results are discussed in general and suggestions offered regarding the relationship between the increase in hydrogen-ion concentration of the blood and the various degenerative changes which occur during larval involution.

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SEXUAL PHASES IN THE AMERICAN OYSTER (OSTREA VIRGINICA)

W. R. COE

OSBORN ZOÖLOGICAL LABORATORY, YALE UNIVERSITY

During the past three years there has been an accumulation of evidence which indicates that in at least three of the so-called dioecious species of the genus *Ostrea* a change of sex frequently occurs from season to season or between early life and full maturity. It may be recalled that at least ten of the more than sixty described species of the genus are regularly hermaphroditic and larviparous. Some of these exhibit a rhythmical sequence of alternating male and female phases, as Spärck (1925) and Orton (1926–27) have so fully described for *O. edulis* and as Coe (1931, 1932) has more recently shown for *O. lurida*.

Moreover, sex determination in other bivalves, as well as in some gasteropod mollusks, has long been known to be in such a labile condition that environmental changes may profoundly alter its expression. It may not be surprising, therefore, to find that changes of sex, especially protandry, as well as various aspects of intersexuality, have been found to occur in dioecious and oviparous species of oysters. For example, Roughley (1928) concluded from his observations on *O. cucullata* that that species, formerly considered dioecious, is regularly protandric, for nearly all the very small individuals were found to be males. An experiment by Amemiya (1929) has been thought to indicate that in *O. gigas* the sexual phase of each individual is determined each winter without influence from its previous sexual condition.

Many years ago Stafford (1913) found indications that the American oyster (*O. virginica*) is protandric on the Canadian coasts and presented evidence that the young animal becomes a sexually mature male when it has reached a length of about 25 mm. At 32 mm. the gonads may be distended with spermatozoa.

More recently Burkenroad (1931) has also shown that protandry occurs in this species on the coast of Louisiana. He concluded that although the sexes of the adults are morphologically separate, each individual is essentially a protandrous hermaphrodite. His evidence indicates that close association with large oysters causes some individuals to assume or retain the male phase, although other oysters of the same size, but growing singly, are predominantly females. He

found small individuals to be almost always males regardless of their associations and interpreted his evidence as indicating that the likelihood of large oysters being males decreases rapidly as the distance from other individuals increases.

Further evidence of protandry in this species has been furnished by Needler (1932) from oysters collected at various localities on the North Atlantic coasts of the United States and Canada. She con-

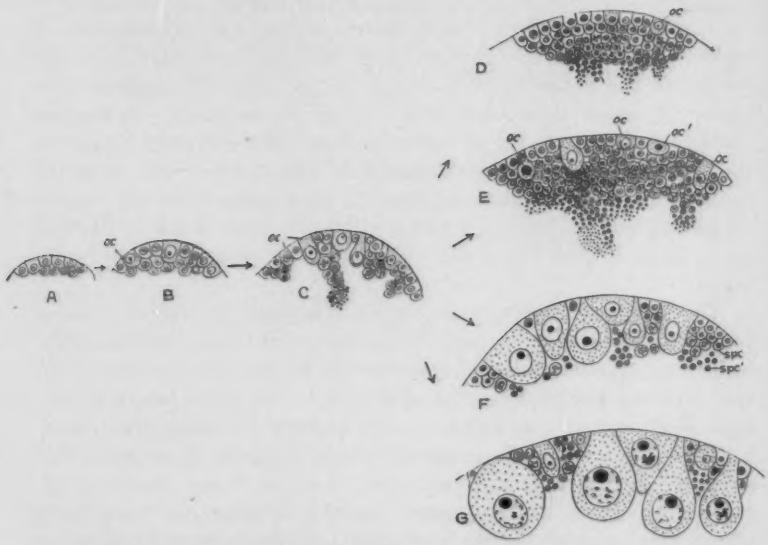

FIG. 1. Diagrams showing the primitive intersexual gonad and its transformation into the definitive spermary and ovary. *A*, early gonad with undifferentiated cells; *B*, intersexual phase, with differentiating ovocytes (*oc*); *C*, later intersexual phase with preliminary abortive spermatogenesis; *D*, spermary with only a few small ovocytes; *E*, spermary with many ovocytes, some of which (*oc'*) are in process of degeneration; *F*, young ovary, with many spermatocytes (*spc*), some of which (*spc'*) are pycnotic; *G*, nearly ripe ovary with residual cells and degenerating spermatocytes.

cludes that the majority of individuals first mature as males and that many of them later change to females. The change may be hastened by favorable nutritive conditions and may possibly be retarded by close association with older females. She observed one instance where a three-year-old male changed into a functional female in the interval preceding the next breeding season.

In order to determine more precisely the sequence of these changes in sexuality and the histological activities which accompany them,

the gonads of a large number of oysters have been examined at frequent intervals during the first two years of their lives. Some of this material was collected from rocks along the shore of Long Island Sound in the vicinity of New Haven; some was taken from various natural oyster beds near Woods Hole, Massachusetts; many samples both of cultivated and untransplanted oysters from Quinnipiac River, New Haven Harbor, and Long Island Sound have been supplied by Mr.

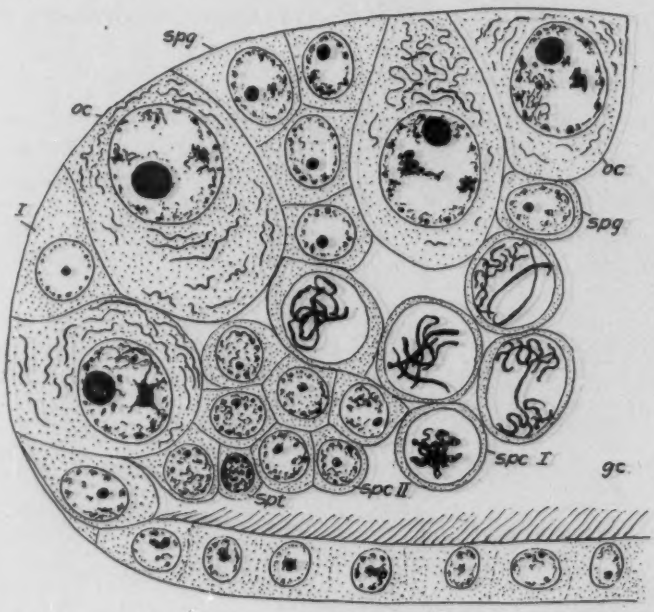

FIG. 2. Primary gonad in animal about four months of age, showing large ovocytes (*oc*), spermatogonia (*spg*), and primary spermatocytes (*spc* I) in spireme phase, with secondary spermatocytes (*spc* II) and a single spermatid (*spt*) bordering the ciliated genital canal (*gc*).

Howard W. Beach, Chairman of the Research Committee of the Oyster Growers and Dealers Association of North America, while a most instructive series of first-year stages was furnished by Mr. J. B. Glancy from the floats of the same Association at West Sayville, Great South Bay, Long Island.

From these collections the early development of the gonads and their transformations in the course of successive phases of sexuality have been studied both in life and by means of serial sections.

Development of the Primary Bisexual Gonads

The profusely branching tubules of the primitive gonads can be found in young oysters at the age of six to eight weeks after setting or when the shell has reached a length of 6–10 mm. They ramify within the thin layer of connective tissue immediately beneath the body walls as Stafford (1913) has previously described.

In these primary gonads the germinal epithelium consists of a thin layer of morphologically undifferentiated cells which lie upon the inner side of the tubular gonad (Fig. 1), while the cells which line the outer

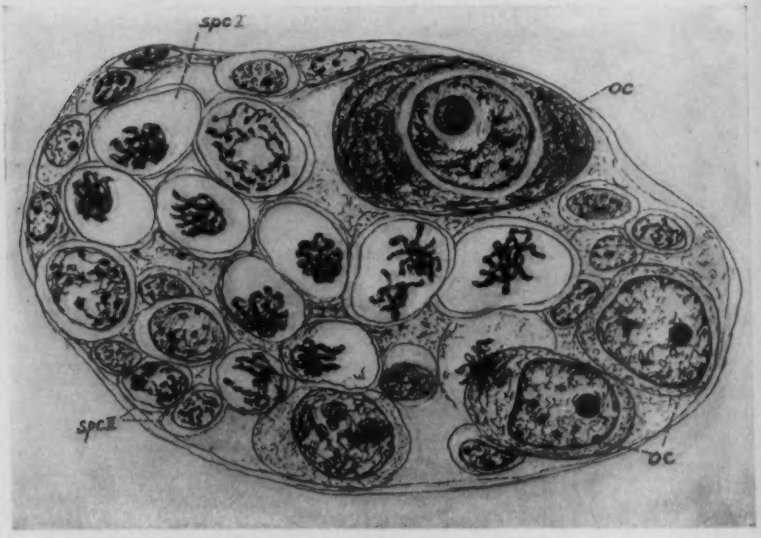

Fig. 3. Primary intersexual gonad, showing primary spermatocytes (*spc* I) and ovocytes in synapsis, secondary spermatocytes (*spc* II) and several large ovocytes (*oc*). Age about four months.

wall, adjacent to the epidermis, become differentiated into the ciliated epithelium of the genital canals (Figs. 2, 4, 5, 6) as Hoek (1883–84) so fully described many years ago for *O. edulis* and as Coe (1932) verified in *O. lurida*.

A few weeks later the germinal epithelium shows a differentiation into larger and smaller cells. The former are soon recognizable as ovocytes by the presence of fibrillar mitochondrial bodies, while many of the latter show by their rapid proliferation and later specialization that they belong to the male germ line.

By the middle of October, at the age of about three months, the shells of some individuals have become 20–25 mm. in length, and in these the gonads have already become distinctly bisexual (Figs. 2–6). A typical section through the genital canal at this age (Fig. 2) shows more or less numerous indifferent cells remaining along the inner border, interspersed with large ovocytes in which the coarse mitochondrial filaments of the so-called yolk nuclei are always con-

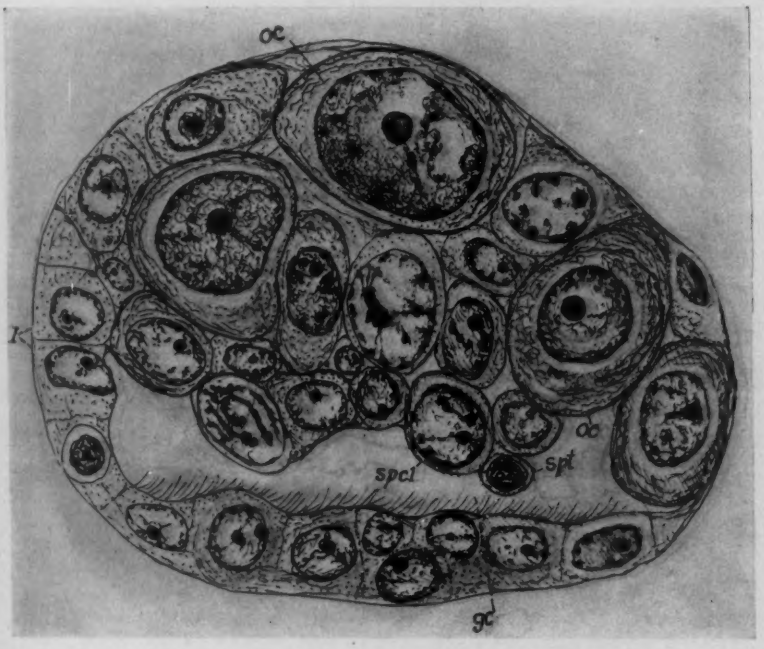

FIG. 4. Primary intersexual gonad in an animal about four months of age, showing indifferent cells (I) and several large ovocytes (*oc*), with spermatocytes (*spc* I) and a single spermatid (*spt*) bordering the ciliated genital canal (*gc*).

spicuous. Morphologically less well differentiated are the ovogonia and spermatogonia which resemble each other so closely that they cannot ordinarily be distinguished. Both types of cells then pass through similar synaptic phases accompanied by spiremes of coarse, densely-staining chromosomes (Figs. 2, 3, 9*B*, 9*C*) as they transform into ovocytes and primary spermatocytes respectively.

The spermatogonia proliferate rapidly, leading to the formation of the numerous spermatocytes which soon give the intersexual gonad

a predominantly male appearance. In some individuals the spermatocytes complete their meiotic divisions to form spermatids, thereby accentuating the resemblance of the gonad to a spermary (Figs. 4–6).

In other animals of the same age the gonads may retain a closer similarity to an ovary (Figs. 2, 3), and it is improbable that any spermatids are formed in every individual. All degrees of inter-

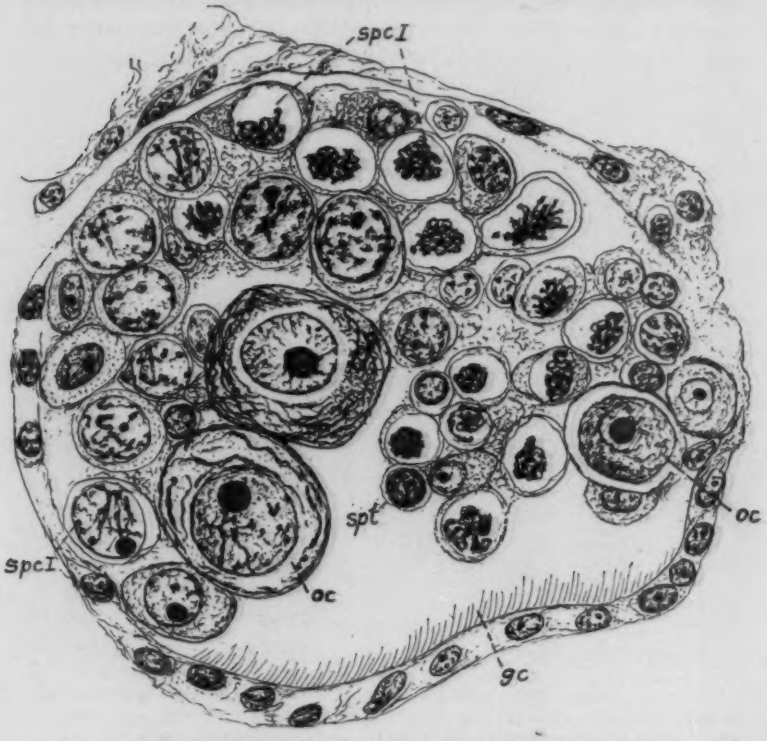

FIG. 5. Primary gonad, with ovocytes (*oc*) and primary spermatocytes (*spc* I) in synapsis; a few spermatids (*spt*) are already present on the border of the genital canal (*gc*); age about four months.

sexuality are found, although the vast majority of individuals are predominantly male. Not infrequently some parts of the system may assume a distinctly male appearance while adjacent follicles are characteristically female, as shown in Fig. 6.

The process of spermatogenesis does not continue through the winter, however, in the localities investigated and no functional

spermatozoa are formed. The gonads may continue to increase somewhat in size during December, with an increase in the numbers of secondary spermatocytes and spermatids. Their activities are then interrupted until there is a rise in the temperature of the water the following spring.

Hibernation

During the long period of hibernation, when the valves of the oyster remain closed, relatively minor changes occur in the primary gonads and these are mainly regressive in character. There is little, if any, increase in the size of the gonads during the oyster's first

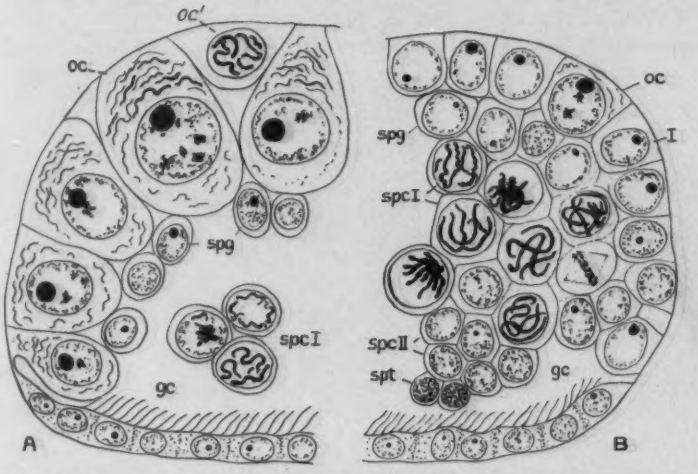

Fig. 6. Portions of two follicles from the same individual at the age of about four months: one of these (*A*) is predominantly of the female type while the other (*B*) consists mainly of spermatogenic cells, although both show some indications of their intersexual character; *oc'*, young ovocyte in spireme phase; other letters as in Fig. 2.

winter or in the numbers of their constituent cells. On the contrary, many of the previously differentiated cells become obviously abnormal and evidences of cytolysis are frequently seen. Remains of disintegrated cells sometimes occur in the lumens of the follicles and ducts.

Transformation to Functional Gonads

At West Sayville the animal retains its primary or immature gonad throughout the winter as the figures in Table I will show. As the water becomes warmer, however, spermatogenesis is resumed in some of the larger animals and in March about one-fourth of the young

oysters examined had transformed into males. No females were recognized until a month later.

In an occasional individual, due apparently to poor nutrition, the primary bisexual type of gonad is retained until the second year, at which time a few functional spermatozoa may be formed.

Males

The transformation of the bisexual gonad into a spermary consists in the proliferation of spermatogonia and spermatocytes, usually accompanied by the disintegration of some of the previously formed ovocytes (Fig. 1). There is a rapid extension of the branching system of follicles, the new branches being, as a rule, exclusively male in appearance, for the reason that the ovocytes usually found in the older

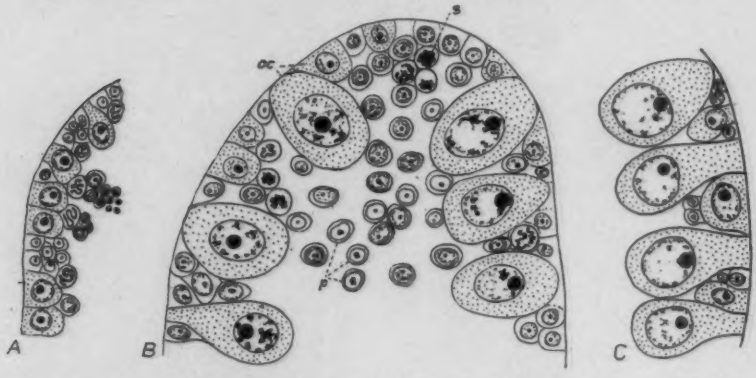

FIG. 7. Transition stages in the development of the mature ovary (*C*) from the primitive intersexual gonad (*A*). *B*, developing ovary in an individual about ten months of age, showing numerous small pycnotic cells (*p*) in the lumen and between the ovocytes (*oc*); *s*, cells in spireme phase.

parts of the system are not carried into the new follicles (Fig. 8). But this rule is not without exceptions, for many grades of intersexuality occur and the gonad is classed as a spermary in this report if it has a distinct preponderance of spermatogenic cells, even though many ovocytes are situated along the walls of the follicles (Fig. 12, *A*). The term hermaphrodite is reserved for those cases in which there are extensive areas or large masses of the cells representing each of the sexes (Fig. 12, *B*).

Females

As the season advances an increasing proportion of the young oysters attain sexual maturity, as shown in Table I. During April

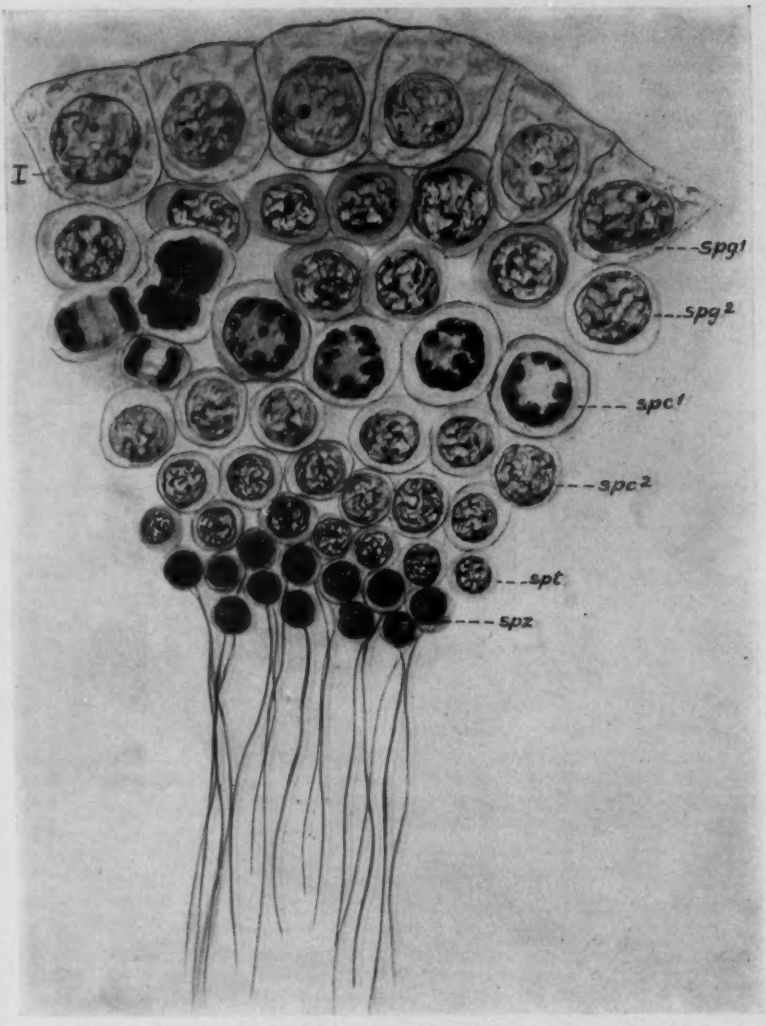

FIG. 8. Mature spermary, showing indifferent residual cells (*I*) and normal spermatogenesis; *spg*^{1}, *spg*^{2}, primary and secondary spermatogonia; *spc*^{1}, *spc*^{2}, primary and secondary spermatocytes; *spt*, spermatids; *spz*, mature spermatozoa.

the first females were found and these showed the transition of the intersexual gland into an ovary (Fig. 7). This process is accomplished by the concurrent growth of the primitive ovocytes, with additions from the small ovogonia, and the pycnosis and eventual cytolysis of such secondary spermatocytes and spermatids as may be present (Fig. 7).

Many indifferent cells and presumably some of the spermatogonia remain as residual cells even after ovulation has occurred. The first crop of eggs discharged is very small, as only a minute proportion of the definitive ovocytes reach maturity until after the first ovulation

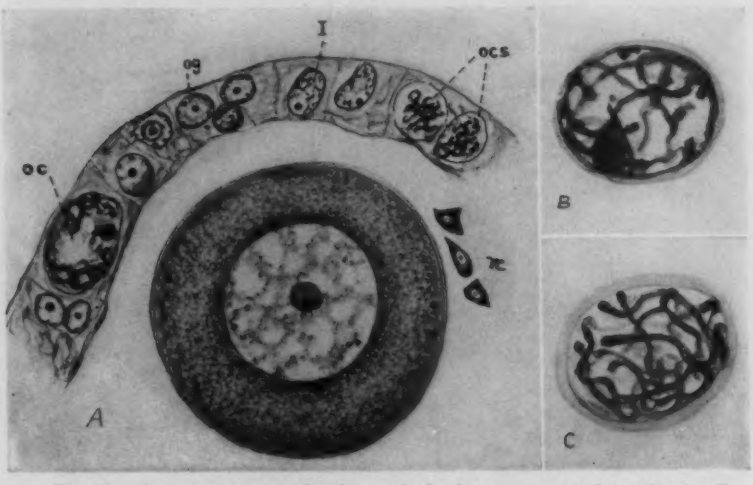

FIG. 9. *A*, mature ovary, showing a single ripe ovum and the residual cells of the follicle; *I*, indifferent residual cells; *og*, ovogonia; *ocs*, young ovocytes in synaptic phase; *oc*, residual ovocyte; several pycnotic residual cells (*rc*) are free in the lumen. *B*, *C*, spireme stages in young ovocytes.

(Fig. 9). The genesis of the ripe ovum, measuring about .05 mm. in diameter has been so fully described that further discussion here is unnecessary. The spawning reactions of both male and female have been fully investigated in this and other species by Prytherch (1924) and by Galtsoff (1932).

Functional Hermaphrodites

True hermaphroditism in the adult American oyster had been considered a rarity until Burkenroad (1931) reported that about 1 per cent of the general population on the coast of Louisiana belonged in this category. Needler (1932) also found about the same per-

centage of hermaphrodites from one locality and a smaller proportion from other areas on the coast of Prince Edward Island. Those that she found were in their third year except one each in their fourth and fifth years.

Although the primary gonad is normally bisexual and at least some degree of intersexuality is usually found in sexually mature yearlings, only 4 among 96 such individuals from W. Sayville and 4 among 389 from New Haven Harbor were classed as true hermaphrodites. These showed approximately equal areas of male and female cells (Fig. 12, *B*). Several others were considered to be hermaphroditic males, being provided with spermaries of normal appearance except for the presence of scattered ovocytes (Fig. 12, *A*), sometimes fully grown.

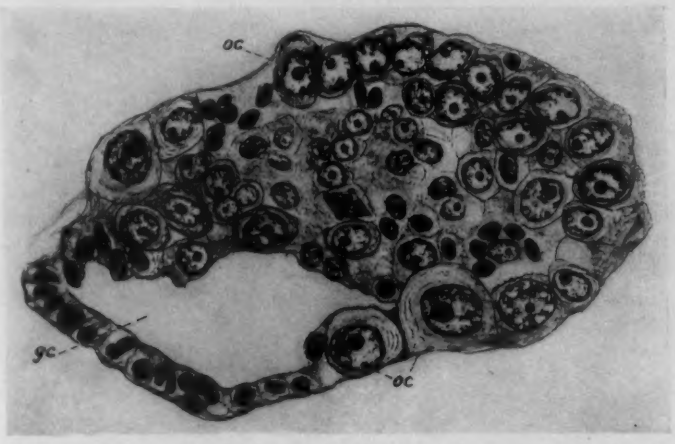

FIG. 10. Spermary early in second year, showing its continued intersexual character; *gc*, genital canal; *oc*, ovocytes.

SELF-FERTILIZATION

Among 55 sexually mature yearlings taken from the culture floats at West Sayville on June 23 were two functional hermaphrodites. Many of the ova in each of these proved capable of self-fertilization and apparently normal development in spite of the vast excess of sperm present. Needler (1932) has reported a similar observation.

SPERMATOGENESIS

The successive stages in the process of spermatogenesis are similar to those described by Coe (1932) for *O. lurida* except that the spermato-

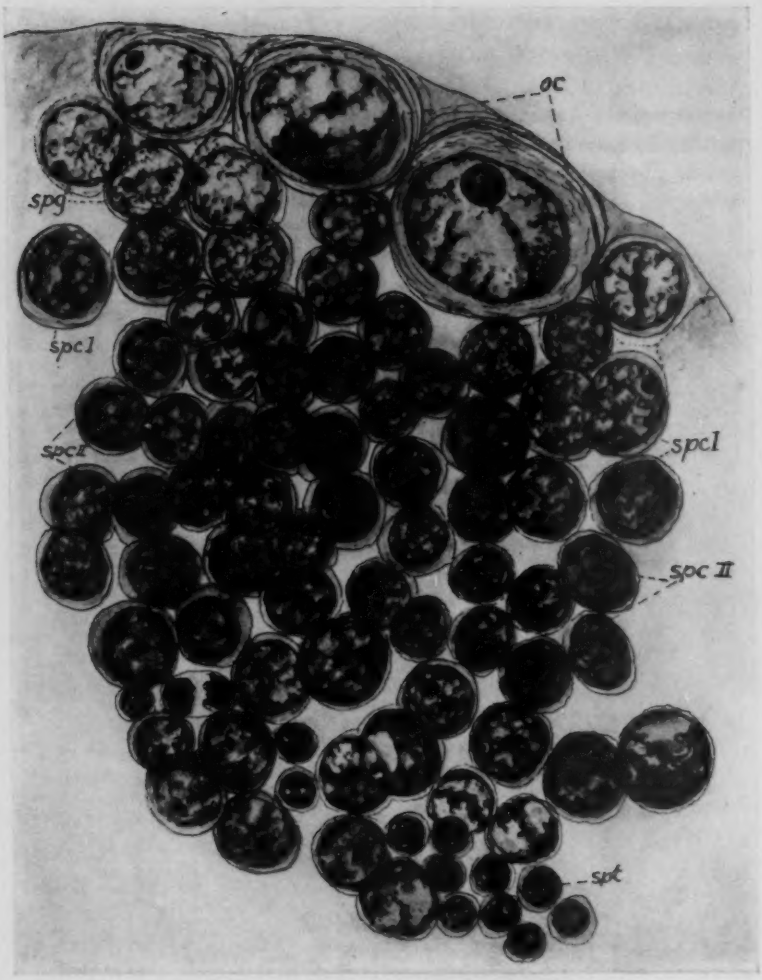

FIG. 11. Spermary at end of second year, showing its continued intersexuality by the presence of large ovocytes (*oc*) on wall of follicle; letters as in Fig. 2.

cytes and spermatids are free to separate immediately after their formation (Fig. 8), instead of adhering in masses as they do in the latter species where the spermatozoa are united into sperm-balls.

Gonads at the End of the Breeding Season and During the Second Year

Such individuals as become sexually mature during their first year produce but a relatively small number of gametes, retaining as residual cells a large proportion of the germinal cells composing the gonads. The residual germinal epithelium of the ovary is similar to that shown in Fig. 9, *A* (except for the absence of the single ripe ovum), while Fig. 10 shows a section of a spermary shortly after activity has been resumed near the beginning of the second year.

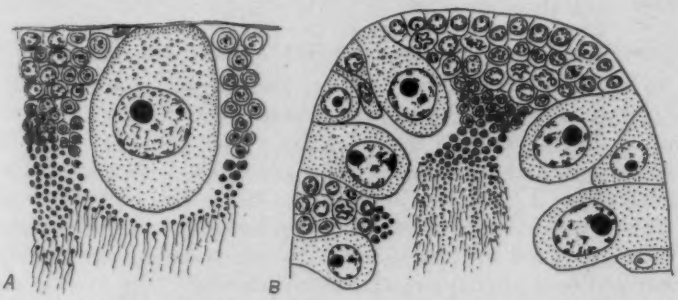

Fig. 12. *A*, partial hermaphroditism; portion of spermary of hermaphroditic male, showing one of the scattered ovocytes. *B*, complete hermaphroditism; gonad with about equal areas of ovarian and spermatogenic tissue. Age about ten months.

The gonads of both sexes after spawning usually retain indications of their continued intersexual character; in the males by the presence of ovocytes along the walls (Figs. 10, 11), and in the females by groups of small cells similar to those shown in Fig. 7. In the latter case, however, there is as yet no proof that these small cells actually belong to the male line, for active spermatogenesis has not been found in an individual classed as a female. A spermary may have few or many ovocytes which sometimes produce yolk in a normal manner, but the reverse conditions seem not to hold except in the relatively small proportion of individuals which exhibit functional hermaphroditism.

Sex Reversal

The observations of both Burkenroad (1931) and Needler (1932) indicate a strong tendency toward protandry in this species. The former concluded that the change to the female phase takes place

when the shell of the young animal has reached a length of about 40 mm., while Needler has positively proved that a change of sex may occur as late as the third or fourth year.

It has hitherto been an open question whether all individuals are protandric; that is, whether the relatively few oysters which spawn as females at the end of their first year may have passed through a preliminary male phase the preceding autumn. It can now be answered that such is not the case in the localities under consideration. The ovary of yearling females develops directly out of the primary intersexual gonad by the growth of the primordial ovocytes and the elimination of spermatocytes and spermatids before spermatogenesis has been completed. The preliminary male phase is thus abortive in these localities although such may not be the case in the warmer areas farther south along the coast.

Correlation of Age, Size, and Sex During the First Year

It has been stated above that at West Sayville, Long Island, a few of the more rapidly growing young oysters become sexually mature as males during March of their first year. Females were first recognized in April and these were mainly among the largest individuals of the group.

Tables I and III show the numbers of individuals of each sex and of each size found at three different periods preceding the breeding season and once after spawning had commenced. The average size of the young females always exceeds that of the sexually mature males of the same age if considerable numbers of each are considered. This rule is shown by Needler (1932) to hold also for oysters at the age of two and of three years, but in still older animals the males are said to average as large as those of the other sex.

The greater size of the females of the younger ages may be correlated (*a*) with a more efficient metabolism associated with the female sex mechanism, or (*b*) greater activity of the female in obtaining food, or (*c*) the actual differentiation of the individual into a female as the result either of its inherent metabolic potentialities or its favorable environmental conditions, or both.

And, conversely, the responsible agency for the determination of maleness and slower growth may be a less favorable metabolism, either genetic or environmental, or, conceivably, the retarding influence of older, associated individuals of either sex.

Inspection of Table I shows clearly that the collection made on March 23 represents a large proportion of oysters that would have become sexually mature later in the season and these would presumably have included both sexes. Omitting this collection and combining

the two groups taken April 29 and May 21 shows (Table II) that shortly before the beginning of the breeding season in June, 41.6 per cent of 149 specimens sent for examination were still immature, while 41 per cent were males, 1.3 per cent hermaphroditic, and 16 per cent were females. By a curious coincidence these figures agree surprisingly closely with those obtained by Needler (1932) from the same locality

TABLE I

Correlation of Size and Sex during First Year, W. Sayville

Length	March 23			April 29			May 21				June 23			
	Im.	M.	F.	Im.	M.	F.	Im.	M.	H.	F.	Im.	M.	H.	F.
mm.														
10–19 ..	6	—	—	16	—	—	4	—	—	—	2	—	—	—
20–29 ..	6	—	—	19	1	—	8	—	—	—	5	3	—	—
30–39 ..	7	1	—	4	12	2	9	14	—	1	2	8	—	—
40–49 ..	7	3	—	1	12	1	1	7	—	1	—	11	1	1
50–59 ..	—	2	—	—	5	10	—	7	2	4	—	7	—	7
60–69 ..	—	1	—	—	—	3	—	3	—	2	—	12	1	1
70–79 ..	—	—	—	—	—	—	—	—	—	—	—	—	—	3
Total...	26	7	0	40	30	16	22	31	2	8	9	41	2	12

TABLE II

Correlation of Size and Sex; W. Sayville. Groups from April 29, May 21, and June 23 combined.

	Total	Im.	p.c.	M.	p.c.	H.	p.c.	F.	p.c.
Less than 40 mm.....	110	69	62.7	38	34.5	0	0	3	2.7
More than 40 mm.....	103	2	2.0	64	62.1	4	4.0	33	32.0
Less than 50 mm.....	146	71	48.6	68	46.6	1	0.9	6	4.1
More than 50 mm.....	67	0	0	34	50.7	3	4.5	30	44.8
Total.....	213	71	33.3	102	47.9	4	2.0	36	17.0

Im., immature; sex not determinable; M., male; H., hermaphrodite; F., female.

at the end of June, 1931. She found 45.3 per cent immature, 38.7 per cent males, and 16 per cent females among 119 individuals which she examined.

On June 23, 1932, however, the writer found that only 14 per cent of the 64 samples studied were still immature, while 64 per cent were males, 3 per cent functional hermaphrodites, and 19 per cent females

(Table I). The smaller proportion of sexually mature yearlings in 1931 as compared with 1932 may be accounted for by the somewhat higher temperature of the water during the growing season of the latter year.

Combining the three groups examined April 29, May 21, and June 23, 1932, numbering 213 individuals, shows 71, or 3.33 per cent, immature; 102, or 47.9 per cent, males; 4, or 2 per cent, functional hermaphrodites; and 36, or 17 per cent, females (Table II).

Considering only the 149 individuals of the present collections that were sexually differentiated we find that they comprise 109 males; 4 hermaphrodites, and 36 females, a percentage ratio of 73.2, 2.7, and 24.2 respectively.

TABLE III

Correlation of Size Groups and Sex First Year; W. Sayville

Length	March 23			April 29			May 21				June 23			
	Im.	M.	F.	Im.	M.	F.	Im.	M.	H.	F.	Im.	M.	H.	F.
mm.														
Less than 40	19	1	0	39	13	2	21	14	0	1	9	11	0	0
More than 40	7	6	0	1	17	14	1	17	2	7	0	30	2	12
Less than 50	26	4	0	40	25	3	22	21	0	2	9	22	1	1
More than 50	0	3	0	0	5	13	0	10	2	6	0	19	1	11
Total	33			86			63				64			

Im., immature; sex not determinable; M. male; H. hermaphrodite; F. female.

At the end of June, 1931, Needler found 46 males and 19 females among the 65 sexually mature individuals which she examined from the same locality. Combining these figures with those of Table II gives a total of 155 males, 4 hermaphrodites, and 55 females, a ratio of nearly 3 males to 1 female.

Of the sexually mature individuals less than 40 mm. in length (Table III) there were 39 males to 3 females, but those of larger size showed 70 males and 33 females. But in the class measuring more than 50 mm. in length (Table III) there are nearly as many females as males, indicating a rather definite correlation of size and sex, as Burkenroad (1931) has shown for the general population and Needler (1932) for the age groups. Probably the most reliable correlation of sex and

size is shown by the collection made during the early part of the breeding season (June 23), representing as nearly as could be estimated an average sample of the entire population remaining on the floats. None of the 12 females was then less than 40 mm. in length and only one of them less than 50 mm. Approximately 27 per cent of the 41 males, on the other hand, measured less than 40 mm. and 54 per cent less than 50 mm. Those in the largest-sized group (70–79 mm.) were all females (Table I). Near the end of the spawning season (July 29) a collection of 70 individuals showed a sex ratio of 100 males to 23 females. Of those measuring less than 50 mm. in length there were 37 males but no females, while those of larger size comprised 15 males and 12 females. Only a single individual was still immature.

The correlation of sex and size is actually much closer than the tables indicate, for the figures given are based upon the length of the shell alone and not upon the actual size of the animal. Selection based on volume would undoubtedly give a still larger percentage of females at the end of the first year in the locality under consideration.

In other areas, however, the sex ratios at the end of the first year are quite different, as Needler (1932) has shown by comparing one-year-old oysters from Prince Edward Island, Long Island Sound, and Great South Bay, Long Island. She concluded that in the colder areas, with a shorter season of activity, relatively few individuals became sexually mature as males before their second year and none as females.

First-year Oysters from Quinnipiac River and New Haven Harbor

Collections were made monthly from September, 1931, to July, 1932, and a very large number of young oysters were examined. Nearly a hundred of these were cut in serial sections. Conditions were unfavorable for a large set in these areas during the summer of 1931 and there was a high mortality of the young oysters in exposed situations during the ensuing winter.

In November, when about four months of age, the shells of the largest individuals were from 20 to 27 mm. in length, but the majority measured only 5 to 15 mm. The gonads of the smaller individuals were beginning to branch out beneath the body walls, while those of the larger animals had already reached the primary bisexual phase, with differentiated ovocytes and spermatocytes (Figs. 2–6). A few spermatids were also present in some individuals.

The rate of growth and the accompanying sexual changes through the ensuing months and until the breeding season in July are summarized in Table IV. The table shows that the first individuals to

become recognizably differentiated sexually were all males, and none of these could be identified with certainty until April or May. During June there was a relatively rapid growth in size, accompanied by an increasing number of sexually differentiated males among the larger individuals. But the correlation of size with sexual differentiation is not without exceptions, as Tables I and IV will show, although the sexual conditions may be definitely controlled by nutrition. Spermatogenesis or ovogenesis may be inaugurated when nutritive conditions are favorable but growth may then be checked by the encroachment of other individuals, while gametogenesis continues. The result may be a dwarfed but sexually functional animal.

TABLE IV

Correlation of Age, Size and Sex, Quinnipiac River and New Haven Harbor

Length	Nov.	Dec.	Mar.	May			July				
	Im.	Im.	Im.	Im.	M.	F.	Im.	M.	H.	F.	Total
mm.											
5– 9	Many	Many	Many	Many	0	0	0	0	0	0	0
10–14	Many	Many	Many	Many	0	0	2	0	0	0	2
15–19	Some	Many	Many	Many	0	0	6	11	0	0	17
20–24	Few	Some	Some	Some	Few	0	9	41	0	0	50
25–29	Few	Few	Few	Few	Few	0	0	104	1	2	107
30–34	0	0	0	Few	Few	0	0	116	1	3	120
35–39	0	0	0	0	0	0	0	82	2	2	86
40–44	0	0	0	0	0	0	0	26	0	3	29
45–49	0	0	0	0	0	0	0	7	0	1	8
50–54	0	0	0	0	0	0	0	2	0	2	4
Total	—	—	—	—	Few	0	17	389	4	13	423

An examination of 423 yearlings taken at random at the height of the breeding season in July indicated that about 96 per cent of the survivors from the set of the previous year had become sexually mature. Most of these were males filled with spermatocytes and ripe spermatozoa (Table IV). The other 4 per cent still retained the primary intersexual gonads and indicated that sexual maturity had been postponed until their second year. All of these immature individuals, as would be expected, showed evidence of unfavorable nutritive conditions as indicated by their dwarfed size.

The predominantly protandric nature of the group is shown by the fact that 389, or nearly 96 per cent of the 406 sexually mature indi-

viduals examined were more or less fully ripe males, while only 13, or 3.2 per cent, were females. Four, or 1 per cent, were functional hermaphrodites with large masses of both ova and spermatozoa. The ratio of males to females is thus 100 : 3.3.

While the number of females is too small to be statistically dependable, it will be observed that their mean length is somewhat greater than that of the males, but less conspicuously so than in the much larger group from West Sayville (Table I). If the volumes had been measured, instead of the lengths, the females would have shown a still greater comparative size.

Comparison of Tables I and IV will show that the proportion of individuals which mature as females during their first year at West Sayville and in New Haven Harbor is about ten times as great in the former locality as in the latter. The size differences of the two populations of yearlings show that not only is there a correlation between rate of growth and sex as concerns individuals but that the proportion of females is several times higher in the locality where the conditions for growth are the more favorable or the growing season longer. There may be some question as to whether the evidence is sufficient to justify the conclusion that metabolic conditions at the time of sexual differentiation actually determine the direction taken by the primary intersexual gonad in its transformation into the functional organ of the first sexual phase. But until further experimental evidence is available it seems to be the most reasonable hypothesis suggested.

First Year Oysters from Near Woods Hole, Massachusetts

Young oysters from small natural beds in the vicinity of Great Harbor, from the shores of the neighboring islands, and from Onset were examined in the summers of 1931 and 1932. In all of these areas growth is slow during the first year and the shell seldom reaches a length exceeding 30 to 35 mm. The more usual length is 6 to 20 mm.

An examination of 389 yearling oysters from these localities showed that 373, or nearly 96 per cent, were still immature or the sex undeterminable, 9 were males, 3 were hermaphrodites and 4 were females. At the age of two years, when most of the young oysters in that region become sexually mature, the ratio of males to females is about one hundred to fifty-five, with approximately two per cent true hermaphrodites. At this age the average size of the females considerably exceeds that of the males, as was also the case in the other areas during the first breeding season.

Comparison with Other Species

The primary bisexuality of *O. virginica* and the development of the definitive gonads, including later sex reversals, are in many respects similar to the series of sexual phases which characterize such strictly hermaphroditic and larviparous species as *O. edulis* (Orton, 1926–27) and *O. lurida* (Coe, 1931, 1932). In both the hermaphroditic and seasonally dioecious types the more rapid proliferation of spermatogonia as compared with the ovocytes soon gives the early gonad its predominantly male characteristics. In the former type, however, all individuals are thought to become functional males before assuming the female phase, while in *O. virginica* from 3 to 30 per cent of the sexually mature young individuals at different localities show only an abortive male phase, the primary gonad developing into an ovary without completed spermatogenesis.

In each type residual cells of both sex lines remain after the first spawning, providing a cellular mechanism which leads in some of the hermaphroditic forms to a series of alternating sexual phases, while in *O. virginica* the sexual changes appear to be more or less facultative, for it is known that in at least some individuals the same sexual phase may be retained for several years. This is presumably true of the great majority of adult oysters under a stable environment.

Evidences of protandry and sex change have been reported in other species of oviparous oysters. Of a large number of very young *O. cucullata* examined by Roughley (1928) all except about 5 per cent were males and he suspected that these exceptions might previously have spawned as males. He found nine functional hermaphrodites. These were thought to represent stages in the transformation of male to female, but it seems more probable that the sexual conditions in that species are not very different from those here described for *O. virginica*, and that the change of sexuality in both species takes place in the interval between two breeding seasons. Among 3000 large adult oysters from thirty different localities he found a sex ratio of 270 females to 100 males.

In *O. angulata* also hermaphroditism occurs occasionally. Amemiya (1925) found two such individuals among 14 males and 59 females. But it is not known whether that species, which is closely allied to *O. virginica*, experiences similar sex changes.

A most interesting type of sex change has been recently reported by Amemiya (1929) for the Japanese oyster (*O. gigas*), previously considered dioecious. In one summer a small hole was made in the shell of each of several hundred oysters and the sex thereby determined. The sexes were then placed in separate cages and returned to

the sea. A year later it was found that oysters of both sexes were present in each cage. It was concluded that about 25 per cent of the females and 60 per cent of the males had changed their sex during the winter. If these conclusions prove to be well founded, Amemiya's hypothesis, that the sex of any individual of that species is determined each winter independently of its previous sexual conditions, will add a new phase to the many variants of sexuality in animals.

Since there is such a wide diversity in the abundance and size of the ovocytes in the sexually mature young males of *O. virginica*, it is pertinent to inquire whether there may not be two genetically distinct types of these males. The samples studied indicate, as shown in Table II, that at West Sayville about 48 per cent of the entire one-year group or more than 70 per cent of the sexually mature yearlings function as males, while in New Haven Harbor there are fully thirty males to one female at their first breeding season (Table IV). It is conceivable that those males with but few and very small ovocytes are genetically "true males" while those with more numerous and larger ovocytes may represent the protandric males. Perhaps it is only the latter that later undergo sex reversal, as Orton (1928) has suggested for *Patella*. It would probably be unwise to speculate further in this connection until more complete evidence is available. The appearance of the primary gonads, however, and the changes which they subsequently undergo, suggest that sexuality in this species may rest upon a basis somewhat comparable with that which Witschi (1932) has found in certain races of frogs.

Summary

1. Examination of the developing gonads of young oysters from various localities at frequent intervals during the first two years of life shows that a primary bisexual gonad is formed in each individual within a few months after setting.

2. The activities of the gonad depend upon the temperature of the water and apparently other conditions of nutrition, a much larger proportion of the animals becoming sexually mature during the first year in warmer than in cooler localities.

3. The primary gonad contains the antecedent cells of both sexes, with ovocytes upon the walls of the follicles and spermatocytes intermingled and bordering the lumens.

4. The protandric nature of the primary gonad frequently becomes manifest by the rapid proliferation of the spermatogonia and the formation of primary spermatocytes; the latter soon pass through the synaptic phases and lead to the production of secondary spermatocytes

and spermatids at the age of a few months. But no functional spermatozoa have been observed until the following spring in the areas investigated. The species is not strictly protandric, however, for 3 to 30 per cent of the sexually mature yearlings are females, the ovaries of which have developed directly from the primary gonads without the completion of a preliminary functional male phase.

5. The definitive sexual gland is a transformation of the primary gonad by the proliferation of spermatogonia and the disintegration of many of the ovocytes to form the spermary or, less frequently, the growth of ovocytes, accompanied by the disintegration of spermatocytes and such spermatids as may be present, to form an ovary. But the intersexual character is usually retained to at least some extent in both types of gonads.

6. The proportion of male and female cells in the mature gonad is highly variable, a few large ovocytes being frequently found in some parts of otherwise typical spermaries, while in the ovary some follicles may retain characteristic male cells. True hermaphroditism was found in 1 to 4 per cent of the sexually mature oysters at the end of their first year. Apparently normal development follows self-fertilization.

7. In the warmer of the two principal localities investigated about 70 to 80 per cent of the oysters which became sexually mature during their first year were males. In the cooler locality the proportion of males exceeded 95 per cent.

8. Most of the relatively small number of females are among the largest of their age group, indicating a close correlation between sex and size. This may imply that the female is metabolically the more active sex or that she requires better nutritive conditions in order to mature, or that sex in this species is so labile that the nutritive conditions of the individual at the critical period of sex differentiation determine which of the alternative types of cells in the primary bisexual gonad shall predominate. Alternative genetical explanations are discussed.

9. After the animal has spawned as male or female the gonad may still retain its bisexual character; the sexual phase during the following year may again depend largely upon nutritive conditions.

10. The primary bisexuality of this species and the cellular mechanism for sex reversal here reported are interpreted with reference to related species of the genus in which hermaphroditism and alternating sexual phases have been retained.

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A COMPARISON OF THE LIFE HISTORIES OF MICTIC AND AMICTIC FEMALES IN THE ROTIFER, HYDATINA SENTA[1]

JOSEPHINE CAROLYN FERRIS

DEPARTMENT OF ZOÖLOGY, UNIVERSITY OF NEBRASKA

INTRODUCTION

The life history of the rotifer, *Hydatina senta*, involves the reproduction of two kinds of females, amictic females, those which reproduce wholly by parthenogenesis, and mictic females which reproduce parthenogenetically or bisexually.

A comparison of these two types of females in regard to the periods of their life histories was made in an endeavor to find if there is a difference in the metabolic rate of the two and to discover its possible relation to the factors which regulate the production of these two kinds of females.

Miller found that the three types of individuals, amictic females, mictic females, and males of *Lecane inermis* differed in the length of the total life period. The unfertilized mictic females and amictic females not only differ in length of life but also differ in rate of production, duration of fecund and post-fecund periods, and in the degree of correlation between fecundity and length of life.

Miller states that the difference in length of life of these two kinds of females is due to the fact that egg-production is a less strenuous process in mictic females than in the amictic ones and consequently the mictic females survive the fecund period and pass entirely through the post-fecund, dying a natural death in old age. The mictic females produce fewer and smaller eggs than the amictic females at a slower rate and they cease egg-production at an earlier age. The differences in length of life would be due probably to the differences in the metabolic rate in the fecund period.

Jennings and Lynch point out that in the case of *Proales sordida* there is no correlation between length of life and the number of eggs produced in the amictic females but that diversities in fecundity were due rather to the size of the eggs from which they have hatched. Smaller eggs which are supposed to have been produced early in the family produce less fecund individual daughters than the larger eggs produced later.

[1] Studies from the Zoölogical Laboratory, University of Nebraska, No. 172.

Like *Proales sordida* and *Lecane inermis*, there are four distinct periods which can be distinguished in the history of the individual in *Hydatina senta:* (1) the hatching or embryonic period; (2) the prefecund or adolescent period of rapid growth; (3) the fecund or egg-laying period; (4) the post-fecund or old age period.

Hydatina senta is one of the larger rotifers commonly found in stagnant and foul ponds. It has been worked with a great deal in laboratories because it is easily cultivated, hardy, multiplies rapidly and has sexual and parthenogenetic generations. The females used in the present work were taken from a general culture which had been collected from a goldfish pool at Seward, Nebraska, in May 1931.

The experiments and observations were made at the suggestion and under the supervision of Professor D. D. Whitney to whom the author wishes to express her indebtedness for advice and assistance given.

Materials and Culture Methods

Hydatina senta is easily cultured in a variety of solutions. A very favorable one is made by using old hay tea as a basis. This is prepared by boiling 1 gram of ground timothy hay in 4000 cc. of tap water for 10 minutes. It is then strained and allowed to age for 4–6 weeks before using. The tap water from which it was made was placed in direct sunlight for several hours to remove an objectionable amount of chlorine. To 100 cc. of this aged hay tea there was added 1 cc. of 1 per cent urea solution, 1 cc. of 1 per cent ox-gall solution and 1 cc. blood solution. This combination of ingredients made a very favorable culture medium for these rotifers.

The urea stock solution consisted of 1 gram of urea crystals dissolved in 100 cc. of tap water and brought to a boil; the ox-gall solution was prepared by using 1 gram of dried ox-gall plus 100 cc. of tap water and brought to a boil. The blood solution was prepared by using 1 gram of dried blood plus 100 cc. of tap water, brought to a boil and filtered.

Throughout the experiment a pure culture of the flagellate, *Polytoma*, was used as the food. This was prepared by using 1200 cc. of tap water that had been boiled, cooled, and put into a small battery jar. Into this was placed a muslin bag containing 200 grams of bone meal which previously had been brought to a boil and allowed to cool. A fresh hay tea solution also was added which was prepared by boiling for 10 minutes 1 gram of ground timothy hay in 100 cc. of sunned water. This culture was inoculated with *Polytoma* and placed at room temperature in a north light exposure. To maintain a good culture of *Polytoma*, the bag of bone meal was changed every 48 hours and fresh

hay tea solution added. After a few days, however, the culture water would become too foul and develop a red coating of bacterial growth on the walls of the jar. Whenever this occurred a new culture was made by pouring the top of the old culture into another sterilized jar and adding enough sterilized water to make 1200 cc. Then fresh bone meal and fresh hay tea solution were added as stated above. In this manner a vigorous culture of *Polytoma* was maintained for many months.

Food for the rotifers from this culture was prepared daily by removing the film from the surface with a sterilized spoon and thus obtaining *Polytoma* in countless numbers. These were then washed twice with old hay tea solution by means of the centrifuge. One cubic centimeter of this concentrated *Polytoma* was diluted with 15 cc. of old hay tea solution. One drop of this was then placed daily in each individual watch-glass containing one female in 7 cc. of culture solution.

The life histories of 184 amictic, 113 mictic females, and 88 mictic females whose eggs had been fertilized were studied and compared.

The females were all cultivated under similar conditions and kept at a constant temperature of 16° to 17° C. This temperature was maintained by use of a double-walled temperature bath through which there was a continual in- and out-flow of tap water. During the winter months the temperature of this bath was kept quite constant and the entire observations were made during this period.

The record for the life history periods began at the time of isolation of the eggs. This was done by isolating in a container a group of mothers that were about ready to lay eggs. They were given a great deal of food and then observed every hour. The first lot of eggs was not recorded due to the fact that some of them may have been laid previously and have been isolated with the mothers. However, beginning at the end of the first hour after they had begun to lay, the eggs were isolated every hour, placed in a container, and labelled. In this way it was known that the eggs isolated at any particular time had been produced during the preceding hour. These eggs were all placed in the temperature bath and carefully observed on the following day for the hatching of the young females. Upon hatching, the young females were immediately isolated and each placed in a separate Syracuse watch-glass with fresh culture solution. Observations were made twice a day on these young females and an effort was made to obtain within a few hours the hatching-time of the first offspring of each female. Thereafter throughout the experiment each individual was looked at twice daily and observations made.

During the fecund period the offspring of each female were removed twice daily. This was done so that if at the first counting any were overlooked they would be found and removed at the second.

In the mode tables, the individuals were arranged in groups of 5's or 10's for convenience and to save space. The means used, however, in the calculation of the standard deviation and coefficient of variability were obtained from all the individuals which had been carried out to the second decimal place.

General Life History

The non-sexual or amictic females multiply exclusively by parthenogenesis. Their eggs carry the diploid number of chromosomes, are not capable of fertilization, and produce females, thus multiplication by diploid parthenogenesis is carried on for many generations. However, at times from these eggs another kind of female hatches which produces small eggs that develop into males. These male-producing eggs carry the haploid number of chromosomes. The females which produce them are called mictic and are identical with the amictic females in outward appearance. Their eggs, however, are capable of being fertilized. If the eggs of the mictic females are fertilized, they produce instead of the haploid egg a larger, dark, thick-shelled egg which has the diploid number of chromosomes. This winter egg, as it is called, always develops into a parthenogenetic amictic female.

The entire life of a female lasts usually about seven to eight days depending upon the temperature and other variable external conditions. The first swimming offspring appear from 32 to 57 hours after hatching of the mother. To all outward appearance, the mictic and amictic females of *Hydatina senta* are indistinguishable, but they differ markedly in certain features of their life histories, particularly in the periods of fecundity and the total length of life.

Embryonic Period

The hatching or the embryonic period lasts from the deposition of the egg until hatching. During this period embryonic development is taking place. The length of this period varies from 18 to 26 hours with the mean for 184 amictic females at 22.34 hours, for 113 unfertilized mictic females at 22.53 hours, and for 88 fertilized females at 21.40 hours. This shows that there is in all probability no difference in the hatching time. (See Table I.)

Pre-fecund Period

The pre-fecund or adolescent period is one of rapid growth. This period extends from the hatching of the egg to the beginning of the fecundity period as shown by the production of the first egg. The length of this period at a temperature of 16° to 17° C. varies from 32 to 57 hours with the mean for 184 amictic females at 41.66 hours, for 110 mictic females at 42.72 hours, for 80 mictic females whose eggs have been fertilized at 46.91 hours. (See Table II.) There was no mortality during this period of immaturity of the 385 individuals studied.

Fecund Period

During the fecund or egg-laying period the eggs are laid one by one. The first were laid from 32 to 57 hours after hatching. After

Table I

Comparison of the embryonic or hatching period of amictic, unfertilized mictic, and fertilized mictic females.

Number of hours	17	18	19	20	21	22	23	24	25	26	Total
Amictic females	2	9	1	110	81	57	76	127	10	20	493
Unfertilized mictic females	0	5	1	18	18	7	24	23	3	14	113
Fertilized mictic females	0	4	0	18	33	13	11	5	3	1	88

	Mean	Standard deviation	Coefficient of variability	Mode
Amictic females	22.34±.5105	3.1668±.0381	7.96±.1706	24
Unfertilized mictic females	22.53±.1355	2.13 ±.0957	9.49±.4266	23
Fertilized mictic females	21.40±.1126	1.57 ±.0795	7.33±.3615	21

the first egg, additional eggs were produced at varying intervals and this continued for a number of days. The parthenogenetic mictic female deposited 16 to 56 of the small male-producing eggs. The amictic female deposited 16 to 66 of the larger, female-producing eggs. The mictic female whose eggs had been fertilized deposited 3 to 26 of the large, thick-shelled fertilized eggs. The modal fecundity of 184 amictic females is 51 daughters and the mean 45.39 ± .3261. The modal fecundity for 110 mictic females is 46 male young and the mean 42.52 ± .5288. The modal fecundity for 88 mictic females whose eggs have been fertilized is 13 fertilized eggs and the mean 9.98 ± .3015. Thus the mictic and amictic females produce nearly the same mean number of offspring; 42.52 and 45.39 respectively. (See Table IV.) These results are much more nearly in agreement

TABLE II

Comparison of the pre-fecund periods of amictic, unfertilized mictic, and fertilized mictic females

Number of hours	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
Amictic females		1	6	7	4	13	24	3	2	35	11	22	11	5	25	6	3	0	1	0	0	1	0	1	2	1	0
Unfertilized mictic females	4	6	3	2	4	2	0	0	0	17	15	14	10	4	14	4	2	0	1	0	0	0	2	2	1	2	1
Fertilized mictic females	3	0	0	0	0	0	0	0	0	0	1	1	4	4	12	19	8	15	11	0	0	2	0	0	0	0	0

	Mean	Standard deviation	Coefficient of variability	Mode	Total
Amictic females	41.66±.2154	4.333±.1524	10.4±.3642	41	184
Unfertilized mictic females	42.72±.5166	8.047±.3662	18.83±.8579	41	110
Fertilized mictic females	46.91±.2671	3.56 ±.1902	8.29±.4438	47	80

TABLE III

Duration of the period of fecundity of amictic, unfertilized mictic, and fertilized mictic females.

Number of hours	8	13	18	23	28	33	38	43	48	53	58	63	68	73	78	83	88
Amictic females	1	0	0	1	0	0	1	1	5	6	10	12	12	7	29	23	20
Number of hours	93	98	103	108	113	118	123	128	133	138	143	148	153	158	163	Total	
Amictic females	17	11	5	7	3	1	2	3	2	0	1	2	0	1	1	184	

Number of hours	11	16	21	26	31	36	41	46	51	56	61	66	71	76	81	86	91	96	101	106	Total
Unfertilized mictic females	2	0	2	2	0	9	9	5	16	19	15	7	2	8	4	4	2	0	0	1	107

Number of hours	2	7	12	17	22	27	32	37	42	47	52	57	62	67	
Fertilized mictic females	1	0	0	0	3	19	0	0	2	4	23	0	0	0	
Number of hours	72	77	82	87	92	97	102	107	112	117	122	127	Total		
Fertilized mictic females	9	9	0	0	1	3	5	0	0	0	0	1	79		

	Means	Mode	Standard deviation	Coefficient of variability
Amictic females	81.25 hrs.±1.125	78 hrs.	22.53± .781	27.72± .978
Unfertilized mictic females	54.35 hrs.±1.106	56 hrs.	17.00± .785	31.2 ±1.441
Fertilized mictic females	52.87 hrs.±1.740	52 hrs.	23.02±1.232	43.54±2.330

than those found by Miller in *Lecane inermis* where the mictic females produced only two-thirds as many offspring as did the amictic females.

The standard deviation for fecundity in 110 mictic females is 8.16 ± .3715 and for the 184 amictic females 6.32 ± .2225. The coefficient of variation for the unfertilized mictic females is 19.22 per cent ± .8761 and for amictic females is 13.92 per cent ± .4931.

The mictic and amictic females mature and deposit the first eggs at about the same time. Since the mictic female produces practically the same number of offspring as the amictic, the fecund period should be the same. The minimum number of hours of the fecund period was

TABLE IV

Comparison of the fecundity of amictic and unfertilized mictic females and the total number of offspring produced during the life time of each of the 184 amictic and 110 unfertilized mictic females. Also showing the number of fertilized eggs produced by each of the 88 fertilized mictic females.

Number of offspring	16	21	26	31	36	41	46	51	56	61	66	Total
Amictic females	1	2	3	0	6	25	59	66	20	1	1	184
Unfertilized mictic females	1	2	5	5	5	14	42	33	3	0	0	110

Number of fertilized eggs	3	8	13	18	23	28	Total
Fertilized mictic females	1	29	47	10	0	1	88

	Mean	Standard deviation	Coefficient of variability	Mode
Amictic females	45.39±.3261	6.32±.2225	13.92±.4931	51
Unfertilized mictic females	42.52±.5288	8.16±.3715	19.22±.8761	46
Fertilized mictic females	9.98±.3015	4.20±.224	42.18±2.138	13

11 for the mictic and 8 for the amictic females. The total number of hours for the mictic ranged from 11 to 106 hours and for the amictic 8 to 163 hours. The mode for the mictics is 56 hours, for the amictics 78 hours. The mean for the fecund period of 107 mictics is 54.35 hours ± 1.106, for the 184 amictics 81.25 hours ± 1.125, and for the 79 mictics whose eggs were fertilized 52.87 hours ± 1.740. The standard deviation is for the amictics 22.53 ± .7819, for the unfertilized mictics 17 ± .7815, and for the mictics whose eggs have been fertilized 23.02 ± 1.232. The coefficient of variation for the amictics is 27.72 ± .9780; for the unfertilized mictics 31.2 ± 1.441; for the mictics whose eggs have been fertilized 43.54 ± 2.330. (See Table III.)

The mictic female requires on the average 26.90 hours less to produce its offspring, although it produces practically the same number

TABLE V

Duration of the post-fecund period of amictic, unfertilized mictic, and fertilized mictic females

Number of hours	5	15	25	35	45	55	65	75	85	95	105	115	125	135	145	155	165	Total
Amictic females	1	11	38	9	22	20	9	13	5	10	10	3	6	1	3	0	2	163

Number of hours	6	16	26	36	46	56	66	76	86	96	106	116	126	136	146	156	166	176	186	196	Total
Unfertilized mictic females	2	5	20	3	10	9	7	10	2	11	6	2	3	1	0	0	0	1	1	1	94

Number of hours	18	28	38	48	58	68	78	88	98	108	118	128	138	148	158	168	178	188	198	Total
Fertilized mictic females	2	25	1	16	2	0	18	1	6	0	0	5	0	1	0	0	0	0	1	78

	Mean	Mode	Standard deviation	Coefficient of variability
Amictic females	53.94 hrs. ± .743	25 hrs.	14.00 ± .524	25.95 ± 1.347
Unfertilized mictic females	59.29 hrs. ± 1.558	26 hrs.	22.40 ± 1.099	37.78 ± 1.719
Fertilized mictic females	56.80 hrs. ± 2.577	28 hrs.	33.63 ± 1.829	59.20 ± 3.020

as the amictic female. This means that the mictic female deposits its small male-producing eggs, on the average, in more rapid succession than the amictic females which produce the larger female-producing eggs. The unfertilized mictic female produces on the average one offspring every 1.2 hours; the amictic female produces on the average one offspring every 1.8 hours; the fertilized egg every 5.2 hours. These figures represent the mean fecundity divided by the mean duration of the fecund period.

Post-fecund Period

The post-fecund period extends from the deposition of the last egg until death of the individual. During the old age period the activities of the female gradually cease, structural degeneration sets in, and death follows usually about the seventh or eighth day. (See Table V.)

In Table V is given the duration of the post-fecund period for all individuals of the amictic and unfertilized mictic females. Seventy-nine amictic females of 184 or 43 per cent died within 36 hours after deposition of the last egg; 37 per cent died within 24 hours, all died within 160 hours. Of the total 163 individuals having a post-fecund period 38 lived 25 hours, which was the mode and the commonest period of death; from 45–55 hours was the next commonest period. The maximum length of the period of old age for the amictics is 160 hours. For the unfertilized mictic it is 192 hours.

The mortality rate reaches one maximal point at about the beginning of the period of old age, perhaps as the result of the exhausting effort of the production of the last eggs. Finally, towards the end of life, it rises to 100 per cent. A large proportion dies immediately after the period of egg-production. But those individuals which pass safely through this period live for some time; in such populations the old females are thick, heavy and sluggish in their movements.

The mictic females cease to deposit eggs earlier, on the average, than the amictic females. Therefore the post-fecund period of the mictic females is extended, being on the average 59.29 hours, while in the amictic it is 53.94 hours.

General Discussion

The amictic and unfertilized mictic females of *Hydatina senta* under controlled conditions differ considerably in length of life, rate of egg production, duration of the fecund and post-fecund period.

In *Hydatina senta*, the difference in the length of life of the amictic and mictic female probably results largely from the differences in the metabolic rates. The amictic females live longer than the mictic females. (See Table VI.)

TABLE VI

Total length of life (from egg to senile adult) of amictic, unfertilized mictic, and fertilized mictic females

Number of hours	87	97	107	117	127	137	147	157	167	177	187	197	207
Number of amictic females	1	0	0	1	4	0	20	16	14	22	8	29	8
Number of hours	217	227	237	247	257	267	277	287	297	307	317	Total	
Number of amictic females	6	14	9	9	4	6	5	5	1	0	2	163	

Number of hours	86	96	106	116	126	136	146	156	166	176	186	
Number of unfertilized mictic females	1	1	1	1	9	2	25	8	12	7	4	
Number of hours	196	206	216	226	236	246	256	266	276	286	296	Total
Number of unfertilized mictic females	11	2	7	9	4	1	1	0	0	0	2	94

Number of hours	77	87	97	107	117	127	137	147	157	167	177	
Number of fertilized mictic females	1	0	2	2	6	1	2	13	0	14	3	
Number of hours	187	197	207	217	227	237	247	257	267	277	287	Total
Number of fertilized mictic females	5	13	0	10	0	3	1	0	3	0	1	78

	Mean	Standard deviation	Coefficient of variability	Mode
Amictic females	192.95±2.194	46.13±.628	23.90 ±.842	197
Unfertilized mictic females	170.53±1.305	19.94±.914	11.106±.509	146
Fertilized mictic females	172.43± .498	6.65±.355	3.85 ±.205	167

In *Lecane inermis*, according to Miller (1931), the difference in the length of life of the amictic and mictic females results largely from the difference in the severity of the process of egg-production, and therefore the mictic females survive the fecund period better. More mictic females live longer because they produce fewer, smaller eggs than the amictic female at a slower rate, and cease egg deposition at an earlier age.

The mictic females of *Hydatina senta* resemble the females of *Lecane inermis* in that some of them live many hours after the cessation of egg production, but the entire length of life of the amictic females covers a longer period than the mictic females, the fecundity period is longer, but the fecundity is practically the same. The relative longevity of the amictic and mictic female of *Hydatina senta* is not correlated with the relative fecundity as in *Lecane inermis*.

Euchlanis triquetra in certain points resembles *Hydatina senta*. According to the observations found by Lehmensick, the amictic and mictic females produce the same number of eggs and live about the same length of time. But the mictic female produces its eggs more rapidly and therefore has a longer post-fecund period. Wesenberg-Lund states (1930) that "if not fertilized, investigations hitherto carried out seem to show that the number of eggs laid by the two sorts of females is almost the same, but that those of the mictic female are laid in a shorter time."

We find that *Hydatina senta* correlates with *Euchlanis triquetra* in that the amictic and mictic females produce practically the same number but that those of the mictic female are laid in a shorter time.

Investigations show that in different species the relative fecundity, length of life, and rate of egg-production of the mictic and amictic female vary greatly. There are probable physiological differences between the two types of females, but as to what the nature of the fundamental differences may be is for further studies to reveal.

Summary

This paper deals with a comparison of the life cycle of the bisexual rotifer, *Hydatina senta*. The three types of individuals, amictic, unfertilized mictic, and fertilized mictic females are compared as to the periods of their life histories.

The mean length of life for the amictic females is 192.95 ± 2.1947 hours; for the unfertilized mictic females 170.43 ± 1.3051 hours; and for the fertilized mictic females 172.43 ± .4983 hours. More than half of the amictic females survive the modal life duration.

The longer life of the amictic females, as compared with the mictic, results from the probable differences in metabolic rate of the

two. The amictic and mictic females produce practically the same number of offspring, the mean fecundity for the amictic is 45.39 ± .3261 and for the unfertilized mictic 42.520 ± .5288.

The mean duration of the fecundity period of the amictic female is 81.25 ± 1.1250 hours with a standard deviation of 22.53 ± .7819 hours and a coefficient of variability of 27.72 per cent. The mean for the unfertilized mictic female is 54.35 ± 1.1061 hours, the standard deviation 17 ± .7851 hours, the coefficient of variability 31.20 per cent.

In summary, the amictic and mictic females produce practically the same number of eggs but those of the mictic female are laid in a shorter time and the amictic female usually lives a longer life. The relative longevity of the amictic and mictic female of *Hydatina senta* is not correlated with the relative fecundity. (See Tables VII and VIII.)

TABLE VII

Comparison of the relation of life duration to fecundity as shown by amictic and unfertilized mictic females.

	Mean length of life	Mean fecundity	Mode length of life	Mode fecundity	Total no. of individuals used
Amictic females	192.95 hours	45.385 offspring	197 hours	51 offspring	184
Unfertilized mictic females	170.53 hours	42.518 offspring	146 hours	46 offspring	110

TABLE VIII

Comparison of the relation of life duration to rate of offspring production showing the mean time required for the production of an offspring. These figures were obtained by dividing the mean fecundity by the mean duration of the fecund period.

	Mean length of life	Mean rate	Total no. of individuals used
Amictic females	192.95 hours	1.8 hours	184
Unfertilized mictic females	170.53 hours	1.2 hours	110
Fertilized mictic females	172.43 hours	5.2 hours	88

The mictic females of this species may be fertilized during immaturity. The fecundity of the mictic female is reduced by fertilization; the mean number of eggs being 9.97 ± .3015, standard deviation 4.20 ± .224, and the coefficient of variability 42.18 ± 2.1388. The length of life of the mictic female is not appreciably altered by the production of fertilized eggs. The mean length of life for the fertilized mictic is 172.43 ± .4983 hours and for the unfertilized mictic females 170.53 ± 1.3051 hours.

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THE RATE OF OVIPOSITION IN THE FRUIT FLY, DROSOPHILA

HERBERT SHAPIRO

(From the Zoölogical Laboratory, Columbia University, and the Marine Biological Laboratory, Woods Hole, Mass.)

As a subject attracting interest because of economic applications, egg-laying has been studied in the house fly and in other insects with a view to determining the factors affecting the total number of ova deposited. Because of its possible bearing on a question which was formerly under investigation by the author, an examination of the rate of egg production in the fruit fly *Drosophila melanogaster* was undertaken for the determination of individual variations, and the similarities, if any, among different mutants. It is not intended in this account to go into the possible experimental modification of oviposition rate nor to add anything to a treatment of the external influences affecting oviposition in insects in general (Richardson, 1925) but rather to describe the process as found under what may be called "normal" conditions for the life of this genus. Not only because of easy cultivation in the laboratory, eliminating special vivaria as may be required for other insects, but also due to the variety of effective media available (Pearl, 1926; Bridges, 1932) and the small amount of space necessary, *Drosophila* is a form very suitable for the study of egg-laying. Of greater moment is the fact that *Drosophila* lays eggs regularly over a longer period and in larger numbers as compared with the activity of certain other forms in these respects; the Cecropia moth, for example, requires only three or four days to lay its quota of several hundred eggs (Rau, 1910). An interesting and compact account of the biology of the pomace fly and complete references up to 1925 are contained in the monograph of Morgan, Bridges, and Sturtevant (1925); for this reason only a few experiments bearing on this study need be cited.

Castle and associates (1906) demonstrated that close inbreeding did not diminish the fertility of *Drosophila*, provided fertile pairs were selected to continue the stock. For a detailed account of the morphology of the ovary and the internal phenomena of reproduction, reference may be made to the description of Nonidez (1920), and of Laurinat (1931), while the courtship and other external phenomena of reproduction have been observed by Sturtevant (1915, 1921). That factors for egg size exist in all four linkage groups has been brought

out by Warren (1924), who also gives a description of the external appearance of the egg. The cytological events accompanying and consequent upon fertilization have been pictured by Huettner (1924).

Before proceeding further, it might be said that the general result of this investigation is that although the life span, active egg-laying period, and total egg output varied greatly from individual to individual in the various crosses, there is found fundamentally the same method of elaborating eggs, which is followed also in the cases of certain invertebrates other than *Drosophila* for which data are available in the literature, and to which reference will again be made. That is, to put the matter another way, a general equation for the rate of laying may be applied to the data secured.

The search for a means of securing the eggs readily and arranged so that they might be counted offered no real difficulty. It was found that if an agar gel was plated out on a small cardboard spoon about three inches long (such as was used, for example, in the experiments of Patterson, 1929) the cardboard would absorb water, leaving crevices in the surface of the gel. It was particularly desirable to avoid this inasmuch as flies will tend to deposit their eggs in these crevices, thereby rendering difficult the procedure of counting. This defect in the technic was remedied by impregnating the spoons with paraffin of high melting point (68°–70° C.). The medium upon which the flies could live and lay their eggs was prepared by adding 5 per cent of molasses to a hot 1 per cent solution of agar in distilled water. About 175 cc. of this medium would suffice for 110 spoons, into which the medium was poured and allowed to gel, forming a plate which adhered to the spoon, but which might, if desired, be cleanly and easily separated from the spoon in which it was cast. After cooling, a drop of yeast suspension in distilled water was placed on the surface of the "plate," which was tilted to allow the suspension to spread uniformly over the surface. These spoons, made up thus each evening, were then placed in crystallizing dishes, carefully covered to prevent access of foreign flies, and used the following day. The flies were secured soon after hatching, and placed individually in a vial four inches long and an inch in diameter together with one of the spoons. The vial was then plugged with cotton. At approximately 24-hour intervals throughout the life of the fly, the spoon upon the surface of which the eggs were deposited was removed from the vial and a fresh spoon introduced. The eggs could then easily be counted under a binocular. Table I indicates how counts were recorded and includes also some derived data. Frequent examinations in the early stages of the work revealed no deposition of eggs on the walls of the vial or

TABLE I

A sample record. From the data (of an individual of cross *k*) presented in this table, the curves in Figs. 1 to 3 were drawn.

Date (1930)	Hour of removal of eggs	Number of eggs counted	Time elapsed	Total No. of eggs	Time / ln total
			days		
7/16	3:50 P.M.	11	1.00	11	0.4170
7/17	1:58 P.M.	70	1.92	81	0.4369
7/18	2:34 P.M.	102	2.95	183	0.5663
7/19	3:05 P.M.	125	3.98	308	0.6946
7/20	3:02 P.M.	116	4.98	424	0.8232
7/21	2:41 P.M.	148	5.97	572	0.9403
7/22	2:06 P.M.	102	6.96	674	1.069
7/23	2:15 P.M.	136	7.97	810	1.190
7/24	2:17 P.M.	115	8.97	925	1.313
7/25	2:47 P.M.	126	10.00	1051	1.437
7/26	3:06 P.M.	127	11.01	1178	1.557
7/27	3:03 P.M.	124	12.01	1302	1.675
7/28	12:28 P.M.	78	12.90	1380	1.784
7/29	2:51 P.M.	139	14.00	1519	1.911
7/30	2:57 P.M.	92	15.01	1611	2.032
7/31	10:18 A.M.	72	15.81	1683	2.129
8/1	12:03 P.M.	93	16.88	1776	2.256
8/2	10:42 A.M.	98	17.82	1874	2.365
8/3	12:08 P.M.	87	18.88	1961	2.491
8/4	11:38 A.M.	83	19.86	2044	2.606
8/5	11:27 A.M.	61	20.85	2105	2.725
8/6	11:09 A.M.	79	21.84	2184	2.840
8/7	11:25 A.M.	46	22.85	2230	2.964
8/8	11:30 A.M.	57	23.85	2287	3.083
8/9	10:08 A.M.	51	24.79	2339	3.196
8/10	11:11 A.M.	42	25.84	2380	3.324
8/11	12:10 P.M.	30	26.88	2410	3.452
8/12	11:43 A.M.	44	27.86	2454	3.569
8/13	10:46 A.M.	29	28.82	2483	3.687
8/14	11:07 A.M.	41	29.84	2524	3.809
8/15	11:09 A.M.	35	30.84	2559	3.930
8/16	9:41 A.M.	16	31.78	2575	4.047
8/17	11:07 A.M.	25	32.84	2600	4.176
8/18	10:43 A.M.	16	33.82	2616	4.298
8/19	10:50 A.M.	14	34.82	2630	4.421
8/20	10:40 A.M.	11	35.81	2641	4.545
8/21	11:27 A.M.	8	36.84	2649	4.675
8/22	11:04 A.M.	6	37.83	2655	4.798
8/23	11:08 A.M.	7	38.84	2662	4.925
8/24	10:01 A.M.	4	39.79	2666	5.044
8/25	10:43 A.M.	7	40.82	2673	5.173
8/26	10:47 A.M.	2	41.82	2675	5.299
8/27	10:58 A.M.	1	42.83	2676	5.427
8/28	10:20 A.M.	6	43.80	2682	5.548
8/29	11:29 A.M.	2	44.85	2684	5.681
8/30	10:00 A.M.	0	45.78	2684	5.799
8/31	10:28 A.M.	0	46.81	2684	5.929
9/1	11:07 A.M.	0	47.84	2684	6.059
9/2	11:46 A.M.	dead	48.88	2684	6.191

elsewhere on the spoon other than the surface of the gel, so that search for eggs in these locations was not thereafter regularly made. The eggs, being white, and 0.5 mm. in length, stand out clearly against the brown medium. Occasionally recounts were made, and it was found that quite consistently all the eggs would be included in a count. In handling the vials there was a minimum of shaking to avoid any possibility of disturbing the laying activities of the flies. The work was greatly facilitated by the use of an incubator in which the flies were kept at 24° C. ± 1° C.; and for preventing the vials from rolling about, and to maintain the surface of the medium level, by the availability of racks which had been designed by Doctor Calvin B. Bridges, and kindly loaned by him.

The results reported here comprise observations on the complete egg-laying history of some 93 females. Virgins, mated flies, and flies kept with more than one male were followed; while in the case of *Drosophila obscura*, which has been studied extensively by Lancefield (1929), both species or physiological races, Race *A* and Race *B*, were observed. Thus with *Drosophila melanogaster*, these crosses were made:

a. Type (wild) females (virgins).
b. + ♂ × + ♀ (♂ removed after mating).
c. + ♂ × + ♀ (with male).
d. + ♂ × + ♀ (with 2 males. In all cases males were replaced if their death occurred before that of the female.)
e. Vestigial females (virgins).
f. vg ♂ × vg ♀ (with male).
g. + ♂ × vg ♀ (with male).
h. + ♀ × vg ♂ (with male).
i. Sepia ♂ × sepia ♀ (with male).
j. Siblings resulting from cross *h* were mated to each other (with male).
k. Siblings resulting from cross g were mated to each other (with male).
l. Lobe^2 ♂ × Lobe^2 ♀ (with male).

With *D. obscura:*

a. Race *A* ♂ × Race *A* ♀ (with ♂).
b. Race *B* ♂ × Race *B* ♀ (with ♂).
c. Race *A* ♂ × Race *B* ♀ (with ♂).
d. Race *B* ♂ × Race *A* ♀ (with ♂).

A few details of passing interest may be dealt with briefly. After the death of most of the flies the abdomen was opened, the ovaries dissected out, and the number of remaining well-formed eggs counted.

No correlation between this variable and any of the other factors studied could be found. The females varied with respect to their actual egg-laying period, some ceasing to produce eggs several or more days before death, others still laying, though at a much diminished rate, even to the end of the life span. Virgins, as well as mated flies, lay eggs regularly, a fact noted previously both by Hyde (1921) and by Hanson and Ferris (1929). The former remarks, "The fecundity record is apparently not modified as a result of fertilization for the unfertilized female lays eggs regularly and in large numbers," possibly implying that mating has no effect on the rate of laying, a point which will be dealt with again. Complete sterility was found in three flies; two of these contained mature eggs in the abdomen after death. In the third, which had large brown concretions around the spermathecæ, the ventral receptacle and spermathecæ were packed with motionless sperm.

The hybrid females (crosses *j* and *k*) displayed their vigor not only in the increased egg production, which was of the order of two or three times that of the mother or of the females of the stock from which the male parent was selected, but also by their incessant activity in the vials in which they were kept. It was more difficult to replace spoons from these vials than from others, an active fly being unfortunately, quicker than the hand.

In *D. melanogaster* laying commenced in general one to two days after hatching, though this period is prolonged in the case of some of the virgins to seven or eight days. Race *B* of *D. obscura* usually precedes egg-laying by a characteristic period of three days of non-productivity, while Race *A* females wait two days before giving off the first egg.

Figure 1 represents the egg-laying of one of the hybrids of cross *k*—a total of 2,684 eggs in a life of about 49 days. The set of data given by this fly was used also in plotting Figs. 2 and 3. In the first figure, where the total number of eggs is plotted against time, a sigmoid curve results. A similar figure appears for all the other flies, and all the data might indeed be represented in this fashion as a family of curves. For this purpose, however, a more convenient means of plotting is utilized, as will be seen in the last figure. A phenomenon peculiar to *D. obscura* appears also in the curves plotted for them. Most of the females of this genus are in the habit of stopping egg-laying at irregular intervals for a period of one or two days, and very occasionally, longer. This, nevertheless, does not affect the characteristic shape of the curves. These curves, as well as those succeeding, are quite generally representative of the entire group of curves drawn.

One would like to know the appearance of the daily egg-laying curve. Inasmuch as it was not practicable to make measurements at exact 24-hour intervals, and also because a further and more serious objection offers itself in the tendency of flies to lay eggs in irregular spurts, as described by Adolph (1920), who says, "There is a distinct tendency for an individual fly to lay several eggs within a few minutes.

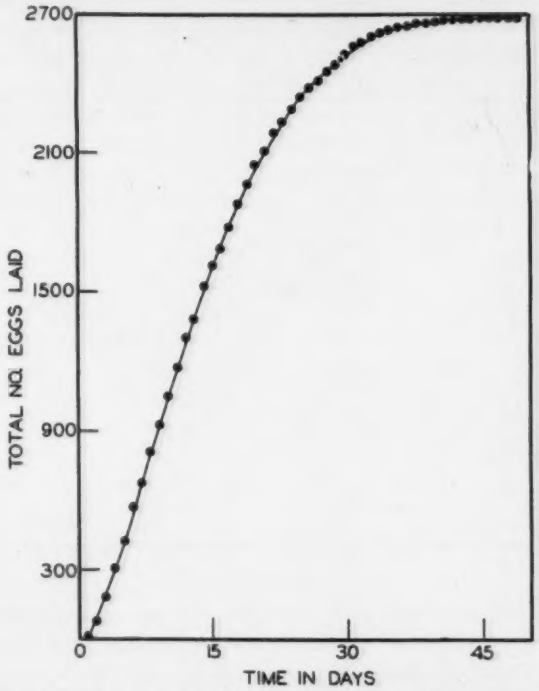

FIG. 1. The total number of eggs produced during the lifetime of a fly. These sigmoid integral curves are obtained also when the data from other individuals are similarly plotted. Figs. 1 to 3 inclusive are curves drawn from the records (contained in Table I) for the same female.

How often such spasms occur under uniformly stimulating conditions is unknown, but there is certainly no daily or weekly periodicity among different individuals," the data were not plotted simply as recorded. Guyénot (1913) remarks, ". . . la ponte se produit sous forme de décharges, causées par la surabondance des oeufs formés." The following means, consequently, were adopted for obtaining the curve shown in Fig. 2, a procedure which may be justified by a simple

hypothetical consideration, inadequate as it may be. The tendency to lay eggs in bunches has been noted. Thus, if at the height of its egg-laying period, a fly laid 120 eggs per day, it would not lay one egg regularly every twelve minutes as might be computed, but rather several eggs during this interval, and then none for perhaps the next half hour. For this reason one must have recourse to an integral curve, for considered from the standpoint of minutes, the egg-laying is irregular, but from the standpoint of days or weeks, it becomes quite regular.

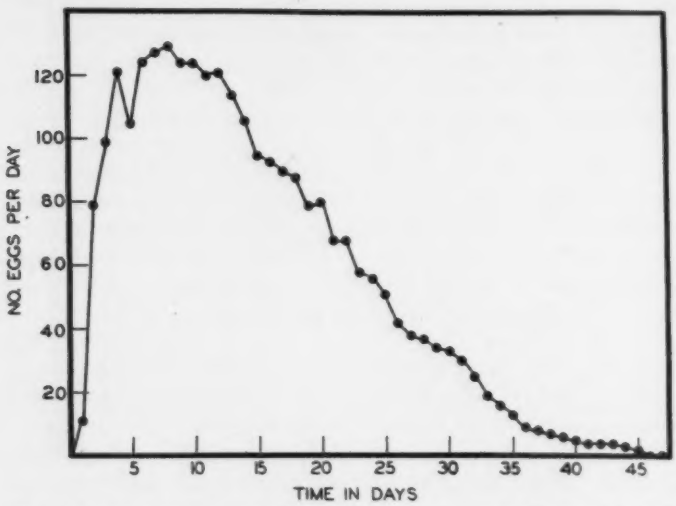

FIG. 2. The daily egg production. This curve was secured by graphical interpolation from an enlarged plot of Fig. 1.

A large integral curve similar to that shown in Fig. 1 was plotted on paper of dimensions $3\frac{1}{2}$ x 4 feet, and then, by use of the mirror tangentimeter described by Latshaw (1925), the number of eggs for each 24-hour interval could be pieced out of the curve with fair precision. The differential curve so secured rises to a maximum of about 130 eggs per day and then falls off gradually to zero at about the end of the fly's life. The area under the curve would represent the total number of eggs deposited. In the attempt to arrive at some general conclusions from the data, the figures were set up in a manner of which Table II is an example. No clean-cut generalizations suggested themselves from a study of such averages. With the apparent general similarity of the type of curve shown in Fig. 1 as drawn for

different individuals in mind, it was decided to study individual cases, to determine whether some general relationship might be found into which they all would fit.

It is found that if $t/\ln T$ is plotted against t, where t represents the time in days at which any given total (T) is attained, the points arrange themselves linearly, as may be seen by inspection of Fig. 3. It will be noted that the last four points are off the curve. This is due to the cessation, on the forty-fifth day, of egg-laying by the fly, which lived four days longer; the point for each of these post-laying days was calculated by using the same total, and these points arrange themselves along another line. The curve shown in Fig. 3 has been drawn also for each of the other 92 females studied and gives an equally good fit for these too, except three others where the points scatter rather more widely on each side of the line. All the sets of data might be

TABLE II

Cross c : $+\sigma^{3} \times + \varphi$ (with male)

Total number of eggs laid	Life span	Day of cessation of egg-laying
	days	
755	27	27
617	20	18
1176	41	28
1537	41	40
830	36	33
1097	35	32
Average 1002±109(A.D.)	35±2.5(A.D.)	29.7±1.2(A.D.)

plotted in somewhat fan-shaped arrangement in the space of Fig. 3, those representing a smaller rate lying above the curve there drawn, those with a greater rate falling below. If instead of this the slope of each curve so obtained be plotted against the grand total of eggs produced, the points fall as found in Fig. 4.

From Fig. 3 it follows that

$$\frac{t}{\ln T} = at + b,$$

where t and T remain with the same meaning indicated previously, a and b representing respectively the slope of the curve and its intercept on the y axis. This yields an equation for the total number of eggs (T) already produced at any stated time (t):

$$T = e^{\frac{t}{at+b}}.$$

To get the change in total with time, the first derivative is taken

$$\frac{dT}{dt} = \frac{be^{\frac{t}{at+b}}}{(at+b)^2},$$

and to arrive at the time at which the rate of egg-laying is at its maximum, the second derivative is taken, set equal to zero, and solved

$$\frac{d^2T}{dt^2} = \frac{be^{\frac{t}{at+b}}[b - 2a(at+b)]}{(at+b)^4},$$

$$t = \frac{b}{2a^2}(1 - 2a)$$

For the fly whose data are plotted as shown in the figures, this maximum is thus calculated to be 5.7 days, when the values used for the constants a and b are 0.121 and 0.221, whereas the value found by inspection of Fig. 2 is about 7.5 days.

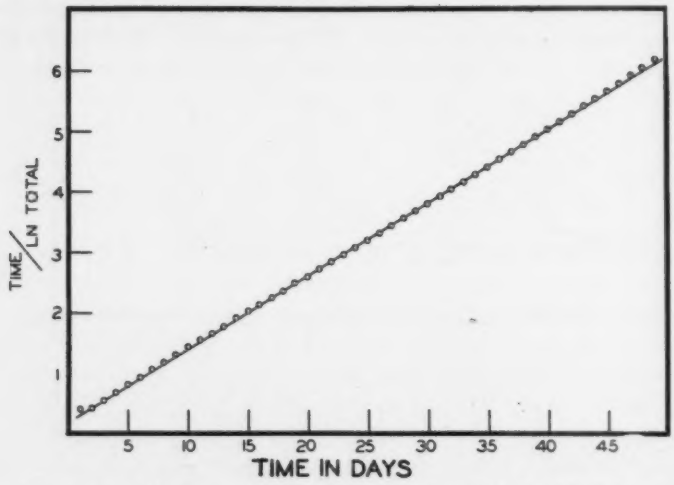

FIG. 3. A typical curve, demonstrating the linear relationship between the variables indicated.

The oviposition data are tabulated in the literature for the cases of some other invertebrates. These have been examined and found to fit the formulation just shown. They are as follows:

Hyde (1921) reported what were considered three unusual cases of fecundity for mated *D. melanogaster* females, namely, totals of 1,613, 1,807, and 2,184 eggs. Similar values have been found quite regularly

in the animals studied here, and with respect to the hybrids it is rather the rule for them to lay 2,000 to 3,400 eggs. Fauré-Fremiet and Garrault (1928) give data for the egg production of *Margaropus australis*, an acarid. While studying the growth of the snail *Lymnæa columella*, Baily (1931) recorded also the egg-laying. The empirical equation applies also to the data presented by these authors, though not quite so well for some of the individuals of the last-mentioned instance as for *Drosophila* due to the more pronouncedly intermittent character of egg production in the snails; however, there is a definite fit. A curve plotted for one of the sets of data presented by each of

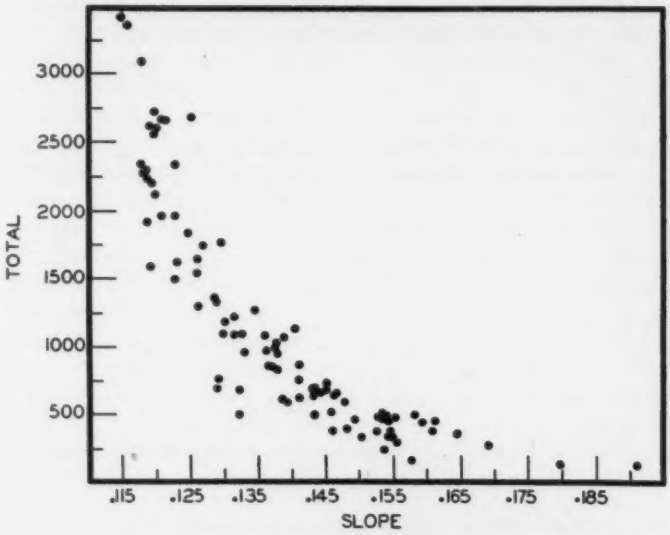

FIG. 4. Each point represents the slope (of a curve plotted as in Fig. 3) in relation to the sum total of eggs produced, for each of the flies studied.

these authors is presented in Fig. 5, where it may be compared, for example, with the curve for the data derived from a female of cross *a* (*D. obscura*).

It is to be pointed out here that it follows from what has been described that unless one is certain that females are producing eggs at the same rate, it is not a sound procedure to select flies at random from a stock and use some for experimentation dealing with egg production, and others for controls, and then to compare the averages of the results. This comparison might possibly lead to erroneous conclusions. From the nature of Fig. 2, it becomes evident that it

should be determined that the shapes of the egg-laying curves for both controls and experimental material, both as regards height of the maximum and length of the curve, are reasonably similar at the outset. In connection with this, an analogous situation may be cited. Davenport (1931) inveighs against the procedure of drawing conclusions concerning growth processes from accumulated data. From the mass statistics of 100,000 children, one might decide that the velocity of growth is greatest at two periods, one in intra-uterine life, and one at about 14.5 years (in the case of the male). When, however, the study

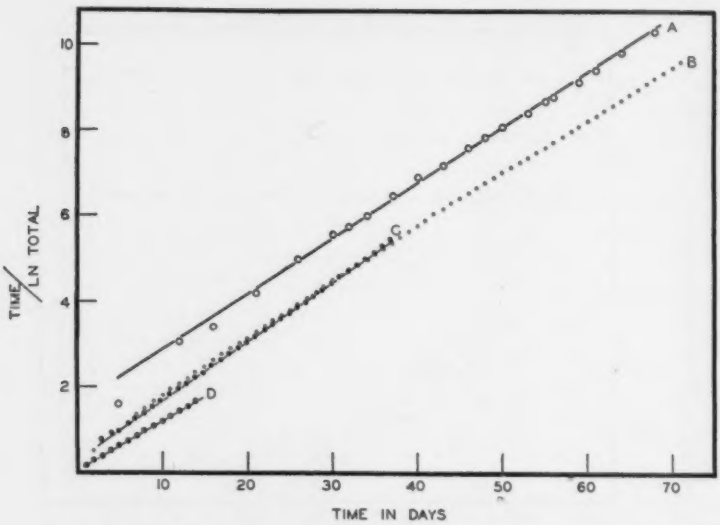

FIG. 5. Comparison of the curves of various invertebrates. *A*, of a snail *Lymnæa columella* (data of Baily); *B*, of *D. melanogaster* (data of Hyde); *C*, of *D, obscura* (Race A); *D*, of a tick, *Margaropus australis* (data of Fauré-Fremiet and Garrault). In *A* the egg output amounted to 725; in *D*, 4346 eggs were produced.

of individual children is made, the resulting curve of growth is found to be very different from that of the mass curves, and varies with different children. Instead of the maximum being reached rather gradually at the age of 14.5 years, a rapid growth of the individual at adolescence, of almost explosive rapidity, is found; the age at which this occurs, and its magnitude, varying with the individual.

When one is dealing with a constantly changing quantity, and where this rate of change will vary for different flies at the same age, the averaging of data will give only a very approximate idea of what

is occurring in general in individual cases. If the proper flies be used, one may see that by averaging different kinds of curves such as are presented in Fig. 2, provided their maxima and duration be different, any kind of average curve, within limits, may be produced. This may perhaps account for the differences observed by Hanson and Ferris (1929), when in one experiment the averaged laying curve for mated white flies rose to a maximum of about 24 eggs per day, while in another similar experiment a maximum of about 52 eggs per day was reached. The maxima of averaged curves may be shifted depending on the nature of the maxima of the individual curves being averaged.

Hanson and Ferris decide from their own data presented as averages that mating results in heightened productivity by the female. There is lack of agreement as to whether or not, in general, the male serves to stimulate an increased production of eggs. Guyénot (1913) states that there is a considerable delay, which varies from case to case, in the initiation of egg-laying by certain *Drosophila* virgins, but that, once commenced, their production gradually approaches in intensity that of the mated individual. In the cases observed by the writer, the vestigial virgins started to lay eggs the second day after hatching, and produced eggs regularly thereafter, whereas type virgins delayed laying from two to eight days after hatching. It is of interest to note that examination of the data shows that certain of the virgins produced more eggs during their life than did mated flies, and this in females from a stock which had been well inbred; although the averages were greater for mated flies than for virgins. These figures, with the deviation of the mean (A.D.), are listed here; the numbers in parentheses represent the number of individuals whose data are averaged.

+ virgins	677 ± 116	(8).
+ ♂ × + ♀ (without male)	718 ± 103	(12).
+ ♂ × + ♀ (with male)	1091 ± 115	(7).
+ ♂ × + ♀ (with two males)	1402 ± 98	(4).

Inasmuch as certain virgins, for example, will lay many more eggs than certain mated flies, one would hesitate to reach the generalization from these averages alone that mating stimulates the female to increased egg production, and cannot help suspecting that the increase may not be an intrinsic one. Eight type virgins laid the following numbers of eggs in the time in days indicated in parentheses: 165 (47), 378 (28), 400 (55), 454 (70), 698 (40), 758 (42), 1088 (70), 1474 (31). In view of the great individual variations, the averaging of the records of much greater numbers of individuals would be required to give a definite statistical answer. It might seem that a means may be

afforded for obtaining an indication of the course of events, in *Drosophila* at least, by use of the equation described above. If mating results in increasing constantly and continuously the rate of egg production, then plotting as in Fig. 3 the data of an experiment where a virgin lays eggs for a given period and is then mated, there should be a break in the line toward the abscissa after the time of mating. Due, however, to the logarithmic nature of the plot, small deviations or changes in the rate do not become readily manifest; consequently this bend is not accentuated sufficiently to permit a definite and unequivocal separation of the parts of the curve that would result.

In an investigation of egg-laying in the domestic fowl, Fauré-Fremiet and Kaufman (1928) advance the interesting hypothesis supported by histological evidence of what is termed a constant probability of transformation of oöcytes, an interpretation entirely independent of the idea of senescence as offered by Brody, Henderson, and Kempster (1923). In seeking a factual basis for this idea, the first-mentioned authors studied the formation of oöcytes in the hen, and the initial number of oöcytes, and proposed an interpretation of the curve of laying according to such data. After a review of the experimental work done on this phase of the subject, it appears that the activity of the germinal epithelium can be restored in certain pathological or experimental conditions (Pearl, 1921), but they conclude that in the hen, under normal conditions, the number of oöcytes is quite limited after birth. Subsequent to birth, oöcytes of the chick grow slowly and progressively. During the period of egg-laying (which may be of eight years' duration) some of the oöcytes undergo a very rapid growth and increase their vitelline mass a hundred-fold in five to eight days, following which they may be laid.

They propose then the following equation, similar to that of Brody and collaborators, to describe the decrease in rate of egg-laying:

$$N_t = N_o e^{-Kt},$$

where N_t = the number of oöcytes still available at time t (reckoned in years), N_o = the initial number of available oöcytes, e = the base of natural logarithms, and K = a constant. The average curve of decrease in a given race in an individual is said then to depend on two values: N_o, the initial number, representing the stock of available oöcytes; the other they term the probability of transformation of oöcytes, a meaning which is attached to the value K, and $1/K$ or θ, would represent the average life of the oöcyte, or the average period during which the oöcytes can remain at the initial state before undergoing the very rapid growth of yolk accumulation. These constants

are supposedly independent of environment, and are probably, according to the authors, hereditary. It is apparent that this equation is formally tantamount to that for a first order reaction, *viz.*,

$$K = \frac{1}{t} \ln \frac{a}{a - x},$$

where the rate of change of the concentration of substance A at any instant is proportional to its concentration at that instant; t represents time, a the original molar concentration, and $(a - x)$ the concentration of A after t minutes. When stated in terms of egg-laying this would mean simply, aside from any implications of a chemically analogous factual basis, that at any instant the rate of decrease of the number of eggs laid is proportional to the number of eggs remaining unlaid.

This equation proposed by Brody, Henderson and Kempster, or the equivalent one of Fauré-Fremiet and Kaufman, for averaged data of egg production by the hen, cannot be carried over to the situation presenting itself in *Drosophila*, where the rise in egg-laying to a maximum is a regular, intrinsic part of the process. The equation of Fauré-Fremiet and Kaufman can be applied only to the descending portion of the curve of laying of the tick *Margaropus australis*, whereas the equation for *Drosophila* can apply also to the entire curve for *Margaropus*. In the case of the arthropod, then, the hypothesis of a probability of transformation of oöcytes appears to be inadequate. To bridge the discrepancy between the curve for laying of *Margaropus* and of the hen, Fauré-Fremiet and Garrault (1928) introduce the conception of the progressive development of a "physiological factor," that is to say, of a complex of somatic conditions allowing yolk accumulation, as being probably responsible for the ascending portion of the curve. Inasmuch as a general equation is found which applies to the whole process, it is perhaps more desirable to conceive it as continuous, and operating throughout as part of the same mechanism, rather than to introduce the idea of a dichotomy, the operation of the second process remaining in abeyance until the completion of the first. However, the existence of such a factor as the first, not yet yielding to exact treatment, is by no means excluded.

Grateful acknowledgment is due Professor D. E. Lancefield and Professor A. H. Sturtevant for reading and criticizing the manuscript.

Summary

The rates of egg-laying of certain mutants of *Drosophila melanogaster* and of two races of *Drosophila obscura* were studied and compared with certain other cases for which data are presented by the authors.

The fecundity of hybrids of *Drosophila melanogaster* was also studied. An empirical equation describes the egg-laying curves of all the flies studied (about 93 in number) and is $T = e^{\frac{t}{at+b}}$ where T represents the total number of eggs already laid at the time t, and e is the base of natural logarithms. The constants a are shown to be correlated with the total number of eggs deposited.

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A NOTE ON THE THYROID GLAND OF THE SWORDFISH (XIPHIAS GLADIUS, L.)

WILLIAM H. F. ADDISON AND MAURICE N. RICHTER

(From the Marine Biological Laboratory, Woods Hole, the Department of Anatomy, University of Pennsylvania, and the Department of Pathology, Columbia University)

In teleosts, the thyroid gland is not so well defined as in elasmobranchs and mammals. As a rule the follicles, in smaller or larger groups, are distributed within an abundance of soft connective tissue, and the boundaries of the thyroid tissue are formed by adjacent structures, not by a distinct capsule. McKenzie (1884) in the siluroid, *Ameiurus*, describes the frame-work as consisting of loose connective tissue which does not form a limiting membrane, but merely passes over into the tissue surrounding the adjacent parts. The thyroid vesicles are scattered through this tissue, showing a tendency to arrange themselves in short rows. Gudernatsch (1911) made an extensive study of the distribution of the thyroid tissue in twenty-nine species of teleosts, belonging to twenty families, and found great variation in the compactness of the tissues, ranging from a complete dispersion of the follicles to a rather compact union of them. Compared with the fishes studied by Gudernatsch, the swordfish, *Xiphias gladius*, is noteworthy among the teleosts because of the concentrated character of the gland and its large size.

In dissecting the heads of swordfish at the Marine Biological Laboratory at Woods Hole, we noticed that the main mass of the thyroid gland formed a large fairly well-circumscribed mass of tissue. It was situated in close relation to the cephalic end of the ventral aorta, and partially encircled it. The color was dark red, due to its great vascularity. Its consistency was moderately soft, but with care it could be separated from the surrounding structures. For convenience in dissecting, it was usually left attached to the ventral aorta.

The general appearance in cross-section and the relation of the thyroid to the ventral aorta are shown in Fig. 1. This specimen was from a medium-sized swordfish weighing 330 pounds after the head, tail, and viscera had been removed. After preservation in 10 per cent formalin the thyroid was a firm mass of a dark gray color. It measured 40 mm. in the sagittal direction and 35 mm. in its widest transverse part. The greatest thickness of the thyroid tissue was 17 mm. The organ was composed, for the most part, of four fairly well-defined

PLATE I

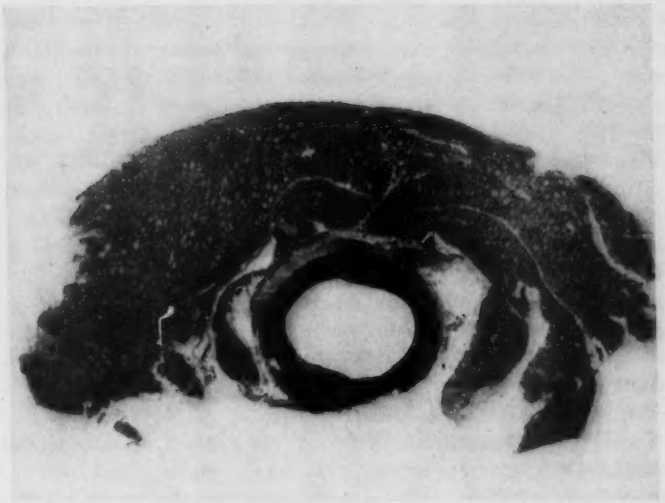

FIG. 1. Cross-section of the thyroid gland of the swordfish partly surrounding the cephalic end of the ventral aorta. × 4.

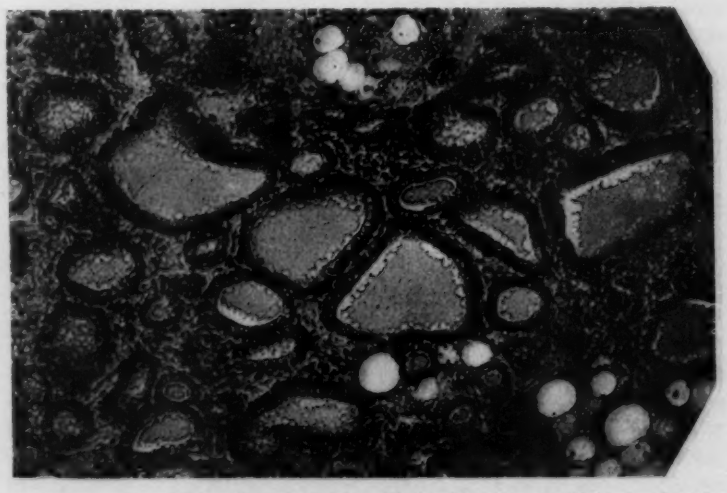

FIG. 2. A small portion of the thyroid gland of the swordfish, showing the compact arrangement of the follicles. × 200.

masses, which were separated from each other by thin connective tissue septa, continuous with the peripheral connective tissue. The two anterior masses, situated on either side of the median line, between the first and second branchial vessels, were larger than the two posterior masses. The latter extended between the second branchial arteries and the single stem for the third and fourth branchial vessels. Each of the four masses consisted of smaller closely-adherent masses or lobules, which were composed of compact thyroid tissue.

Microscopically, the main gland mass shows epithelial-lined follicles containing colloid material (Fig. 2). Around the follicles is a very vascular but scanty supporting tissue in which are a few fat cells. Thus, in certain features, the gland resembles closely elasmobranch and mammalian thyroids. In transverse sections across the entire mass, separate small groups and rows of follicles were also seen. These were scattered in the loose connective tissue between the main part of the organ and the wall of the aorta and the branchial vessel, and showed the typical teleostean arrangement.

The lumina of the follicles vary greatly in size. Of those containing colloid, a small one may measure 30 x 25 μ, a large one 300 x 200μ. The largest ones may measure several millimeters in length. One was found to be 3.5 x 1 mm., and another 2.5 x 0.75 mm. A great number, however, are of intermediate size, measuring about 120 x 80 μ. The general shape is round or oval, the large ones being usually elongated. The walls of the follicles are formed of simple columnar epithelium, which apparently does not rest on a basement membrane.

The epithelial cells are columnar in form, and average 15 x 4.5 μ after fixation in formol-Zenker. The cells are taller and narrower than the "chief" cells of *Mustelus*. According to Ferguson (1911) the latter measure 6–10 μ in height; after fixation in formol-Zenker, however, we find the *Mustelus* cells to be somewhat higher, 13–14 μ. They present many slight variations in shape, some being fusiform, others slightly curved. At places there is a pseudo-stratified appearance, because the nuclei of adjoining cells are alternately higher and lower in position in the cells. The cytoplasm, after staining with Dominici's stain or eosin-azur, is basophilic in the basal portion, and acidophilic towards the lumen. Sometimes the acidophilic zone is seen at the basal margin of the cells. This suggests a reversal of polarity in these cells. In a few cells, the portion of the cytoplasm adjoining the lumen contains acidophile substance in the form of globular masses of varying sizes. These globular masses resemble colloid in appearance. In Gudernatsch's study (1911), the "colloid" cells of Hurthle or of Langendorff were seldom seen. Typical ones

are not seen here. The nuclei are usually situated in the center or in the basal third of the cell-body, and contain relatively little chromophilic substance.

In the follicles one usually finds homogeneous retracted colloid material. Within the colloid material in some, there are lighter staining spherical areas or vacuoles. Frequently, groups of ill-defined epithelial cells are also seen within the colloid, as well as hemorrhagic masses of blood cells. Such follicles often lie deeply in the lobule where they would be well protected from mechanical injury.

Throughout the greater part of the gland, the epithelium is separated from the blood by only the endothelium of the thin-walled vessels. The blood-vessels, however, are numerous, and are of relatively large size. Lymph-vessels containing colloid are also seen. The amount of fibrous tissue between the follicles is very small. In addition to the interfollicular supporting tissue there are wider strands of connective tissue between the lobules. In these interlobular strands are sometimes rows of fat cells and single follicles, or small groups of them. Around the peripheral lobules, the connective tissue fibers are arranged in a parallel manner, somewhat closer together than in the adjoining loose connective tissue. After orcein staining, elastic fibers of small size are demonstrable. These are much thinner than those in the walls of the blood-vessels, and are arranged in a loose network. This peripheral connective tissue could scarcely be regarded as a true capsule, but it is somewhat modified from the ordinary loose connective tissue. The consistency of the organ is thus due more to the close arrangement of the follicles than to the presence of a definite peripheral covering membrane.

Pigment cells are distributed in the loose connective tissue around the organ, and are often perivascular in position, in some places lying against the walls of the blood-vessels.

Studies in the thyroid of fishes have revealed several interesting conditions. In the thyroid of the dogfish, A. T. Cameron (1913) finds by chemical analysis that the iodine content is higher than in that of any mammalian thyroid yet examined. Marine and Lenhart (1911) find that hyperplasia of the thyroid tissue develops in brook trout (*Salvelinus*) kept under certain conditions, and that iodine stops the hyperplasia and causes the thyroid to return to the colloid or resting state. For chemical or physiological studies of the teleostean thyroid it would appear that the swordfish thyroid, on account of its large size and the homogeneous structure of the main part of the organ, would afford satisfactory material.

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INFLUENCE OF HYPOPHYSECTOMY ON THE PANCREATIC DIABETES OF DOGFISH

OSCAR ORIAS[1]

(From the Marine Biological Laboratory, Woods Hole, and the Laboratories of Physiology in the Harvard Medical School)

Using toads and dogs, Houssay and Biasotti (1930*a*, 1930*b*) have shown the marked influence of hypophysectomy on the course of the diabetes produced by pancreatectomy. In their pancreatectomized animals the diabetes was definitely milder when the hypophysis had been removed. It was the purpose of this investigation to determine whether or not the general conclusions reached by these investigators were applicable to a lower form. The smooth dogfish (*Mustelus canis*) was chosen on account of its availability and its position in the vertebrate scale. Furthermore, its cartilaginous skull renders hypophysectomy a comparatively simple procedure.

Pancreas, Hypophysis, and Glycemia in Elasmobranch Fishes

Elasmobranchs have a large pancreas, its tissue being made up of glandular acini and of insular cells functionally equivalent to the islets of Langerhans in the pancreas of mammals (Jackson, 1922). The isolation and removal of the entire pancreas are procedures easily performed without any serious bleeding. Herring (1911) has shown that the hypophysis of elasmobranchs is developed almost entirely from Rathke's pouch. This same investigator (Herring, 1913) states that "the elasmobranch pituitary differs from all other pituitaries in not possessing a posterior lobe. The brain wall of the embryo merely evaginates to form a paired saccus vasculosus, but no pars nervosa is formed." And although de Beer (1926) showed that there is an extension of neuroglia fibers from the border of the infundibular cavity which penetrates the posterior lobe (pars intermedia), it is certainly true that the selachian lacks a true pars nervosa. This means that one has to deal in this form with a hypophysis composed of (*a*) an anterior lobe of eosinophile and basophile cells, (*b*) a posterior lobe (pars intermedia) of basophile cells, and (*c*) a ventral lobe composed also of basophile cells, but containing, in addition, certain curious large cells staining with eosin and of undetermined significance (de Beer, 1926). In the hypophysectomies of the present investigation

[1] Fellow of the Rockefeller Foundation from Argentina.

no attempt was made to discriminate between these various parts. All removals of the gland have been total, and were performed according to the technique described by Lundstrom and Bard (1932).

The normal glycemia in fish has not yet been satisfactorily worked out. All the recent studies dealing with the blood sugar of fishes have shown great discrepancies between the values found not only among specimens of different species, but also among different individuals belonging to the same species. Although no one has been able to determine precisely all the factors responsible for these discrepancies, undoubtedly the variable degrees of asphyxiation involved in the process of obtaining the blood samples for analysis stand as a cause of paramount importance in explaining the widespread variability of results. There is no doubt that asphyxia induces a condition of hyperglycemia which may last during a period of several days even when the fish is replaced in sufficiently oxygenated water. This has been shown by McCormick and Macleod (1925), Simpson (1926), and Kisch (1929). The asphyxial blood-sugar rise is due to a mobilization of glycogen from the liver (Simpson, 1928; Kisch, 1929).

Apart from the influence of asphyxia, the blood-sugar level seems to vary with the different species of fish because of the differences in their habits of life. According to the investigations of Gray and Hall (1930), who studied fifteen species of teleosts, fast-swimming fishes depending on the speed of their movements to catch their prey have a higher blood-sugar level than those less active bottom-feeders that live on crustaceans and other slow-moving creatures.

Before proceeding to the main problem, an attempt was made to determine the normal blood-sugar level of *Mustelus canis*. Blood samples were directly withdrawn from the heart by means of a syringe with the needle inserted through the ventral median line at the anterior edge of the pectoral girdle. As a rule 0.5 cc. of blood was used for each determination. The reducing substances of blood after precipitation of proteins were determined by the Shaffer and Hartmann method (1921) and computed as glucose.

Blood-sugar determinations were performed on each fish used (total of 39) in this work immediately after its arrival in the laboratory from its place of capture in traps situated out in the ocean. The values obtained ranged between 72 and 250 milligrams of glucose per 100 cc. of blood. Sometimes very low values were recorded (from 0 to 50 milligrams per cent), but animals with such low blood-sugar figures were either moribund, or, if apparently normal, they invariably died within a few hours. In this respect our observations entirely agree with those of Scott (1921). After this initial blood-sugar deter-

mination, in order to let the fish recover from the effects of asphyxia and rough handling incident to its capture, it was placed in an immersed floating cage, several meters off shore, exposed to tides and marine currents. After 48 hours, a new sample of blood was withdrawn as quickly as possible and the amount of sugar determined. The figures thus obtained in 10 specimens of *Mustelus canis* ranged between 65 and 137 milligrams of glucose per 100 cc. of blood, the average being 105 ± 5 milligrams, and the standard deviation ± 22 milligrams. No food was given to the animals.

These results, as well as the data found in the literature regarding the blood-sugar level of elasmobranchs, seem to show a clear difference between the amount of glucose in the blood of the species of the genus *Mustelus* (smooth dogfish) as compared with that in the blood of species of the genus *Squalus* (spiny or horned dogfish). In effect, the "weighed average" of all the analyses on fish of the genus *Mustelus* reported by Fandard and Ranc (1914), Scott (1921), Menten (1927) and Fremont-Smith and Dailey (1932), turns out to be 99 milligrams of glucose per 100 cc. of blood, for a total of 34 animals examined. Denis (1922), omitting to say how many animals she investigated, reports for the blood of *Mustelus canis* amounts of glucose ranging from 80 to 181 milligrams per 100 cc., the majority of her results falling between 90 and 110 milligrams.

In so far as the genus *Squalus* is concerned, the "weighed average" of the analyses performed by Claude Bernard (1877), Lang and Macleod (1920), and White (1928), is of the order of 36 milligrams of glucose per 100 cc. of blood for a total of 15 animals examined. It is impossible to account for this difference on the basis of the view that the difference in the blood-sugar levels of these two genera is related to their different habits of life in the way pointed out by Gray and Hall for teleosts. While according to Bigelow and Welsh (1924), the spiny dogfish (*Squalus*) is a strong, fast-swimming animal, the smooth dogfish (*Mustelus*) is a bottom fish, feeding principally on crustaceans. Elasmobranchs of the genera *Torpedo* and *Scyllium*, which according to the analyses of Diamare (1905 and 1906), Diamare and Montuori (1907), and Kisch (1929) have also lower amounts of blood sugar than *Mustelus*, are slow-moving animals of the sea bottom (Couch, 1868).

Pancreatic Diabetes in Fish

Capparelli (1894) was apparently the first to study the effect of pancreatectomy on fish. He removed the pancreas from eels, and was able to find marked glycosuria as a consequence. Diamare (1905, 1906, and 1911), working on elasmobranchs, found considerable

amounts of sugar in the blood of *Scyllium* and *Torpedo* after removal of the pancreas, whereas he was unable to detect any sugar in the blood of these animals before the operation. Probably Diamare and subsequently Diamare and Montuori (1907) failed to find sugar in the normal blood of *Scyllium* and *Torpedo* because of the inadequate methods available at that time, as they themselves suggested. More recently McCormick and Macleod (1925) found in *Myoxocephalus* (sculpin) marked hyperglycemia as a consequence of the ablation of the principal islets, easily removed in this animal, leaving intact the remaining pancreatic tissue. Simpson (1926) working on *Myoxocephalus* and *Ameiurus* confirmed the observations of McCormick and Macleod.

Experiments and Results

Limiting the present investigation to animals of the same species and following as uniform a procedure as possible in handling the animals before, during, and after the operations, it is possible to a certain extent to make "constants" out of the several factors, known and unknown, which modify the blood-sugar level, aside from the experimental conditions created for purpose of the study (pancreatectomy, hypophysectomy, etc.). Finally, a statistical treatment of the data obtained will enable us to get a more complete idea of the significance and validity of the differences between the average values obtained under the different experimental conditions (Dunn, 1929).

Medium-sized animals were chosen (from 70 to 90 centimeters long), regardless of sex, but pregnant females were rejected. Blood-sugar determinations were always performed by Shaffer and Hartmann's method (1921).

The first step of our procedure was always to withdraw a sample of blood from the heart as previously described, for a blood-sugar determination. Then the necessary operations were performed, with care to avoid asphyxia as much as possible. A constant flow of sea water was maintained through the mouth and gills and under these circumstances the respiratory movements proceeded in normal fashion.

The pancreas was removed through an abdominal incision about three centimeters long and the abdominal wall was subsequently closed in layers by silk sutures. The hypophysis was extirpated through a buccal approach. No anesthetic was used. The longest operation lasted about twenty minutes. After they had been operated the animals were treated as previously described.

Forty-eight hours after operation a new sample of blood was secured and another blood-sugar determination performed. The forty-eighth hour after operating proved to be a critical juncture.

TABLE I

Glycemia of Dogfish under Different Experimental Conditions
(Glucose in milligrams per 100 cc. of blood)

OPERATION	Laparotomy		Hypophysectomy		Pancreatectomy		Hypophysectomy and Pancreatectomy		Pancreatectomy and Injury of the Hypothalamus	
	Before operation	After 48 hours	Before operation	After 48 hours	Before operation	After 48 hours	Before operation	After 48 hours	Before operation	After 48 hours
Individual Results	83	150	144	100	185	431	165	155	115	431
	247	125	185	65	144	350	175	328	183	431
	220	115	170	370	144	428	115	267	203	430
	185	185	100	185	205	370	175	370	162	392
	160	105	160	95	124	431	225	205	144	423
	72	105	144	115	195	368	267	395		
	120	185	142	117	155	380	124	349		
	122	226	155	137	135	430	115	215		
					150	430	137	307		
Averages	151±13	149±11	151±6	148±23	159±6	402±7	166±11	288±18	161±10	421±5
Stand. dev.	±52	±42	±23	±90	±26	±32	±49	±77	±30	±15
Prob. error	±35	±28	±15	±60	±17	±21	±33	±52	±20	±10
Differences	2±16		3±23		243±9		122±21		260±11	

Before that time the effects of asphyxia and rough handling inherent in the operations were still too marked, and beyond the 48 hours the mortality began to be rather high.

The animals fall into five groups. The results show respectively the separate effects on blood sugar of (*a*) simple laparotomy, (*b*) hypophysectomy, (*c*) pancreatectomy, (*d*) pancreatectomy and hypophysectomy, and (*e*) pancreatectomy and injury of the hypothalamic region of the brain. The operations were performed in such a way as to permit having animals of different groups simultaneously exposed to the same environmental conditions.

As Table I shows, neither the laparotomy alone, nor the simple hypophysectomy, exerted any significant influence on the glycemia. The values, of course, were above those considered normal, but of the same order of magnitude as the values found before the operation. The removal of the pancreas, as was to be expected, caused a marked increase of blood sugar values: from 159 ± 6 milligrams of glucose per 100 cc. of blood as the average for 9 unoperated animals, to 402 ± 7 milligrams per 100 cc. of blood as an average for the same animals 48 hours after the operation.

When both pancreas and hypophysis had been removed in the course of the same operation, a condition of hyperglycemia also ensued, but the average for this whole group as well as the individual figures were lower than those encountered when the pancreas alone was taken out. That this difference ($- 114 \pm 19$ milligrams) which is certainly significant, is due to the absence of the pituitary body and not to the influence of some direct nervous factor brought into play by the operative traumatism, is demonstrated by the fact that in animals of the fifth group in which the pancreas was removed, leaving intact the hypophysis but injuring the adjacent nervous tissue (hypothalamus), the blood-sugar values were even higher than those found when the pancreas alone was extirpated.

Shortage of time and animals prevented the study of the action of pituitary grafts and the action of pituitary extracts, but the data here reported support the conclusion that in the dogfish (elasmobranch fish) just as in the toad (batracian) or in the dog (mammal) the hypophysis exerts an aggravating influence on pancreatic diabetes, the mechanism of which is still obscure.

Summary

Laparotomy or hypophysectomy does not change the blood-sugar level in the dogfish. Pancreatectomy produces a marked hyperglycemia. The hyperglycemia is, however, less marked if pancrea-

tectomy is accompanied by hypophysectomy, but it is slightly more marked if in addition to pancreatectomy the hypothalamus is injured.

In conclusion I wish to express my indebtedness to Dr. Philip Bard for valuable help and suggestions.

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MELANOPHORES INDUCED BY X-RAY COMPARED WITH THOSE EXISTING IN PATTERNS AS SEEN IN CARASSIUS AURATUS[1]

GEORGE MILTON SMITH

ANATOMICAL LABORATORY, SCHOOL OF MEDICINE, YALE UNIVERSITY

In a recent publication (Smith, 1932) it was shown that if goldfishes (*Carassius auratus*) were exposed to X-rays, an eruption of corial melanophores occurred varying greatly in intensity in different fishes. In some fishes a general cutaneous melanosis resulted, leading even to death of the fish.

Generally speaking, the X-ray eruption of melanophores is a transient affair, appearing about the fifth day after exposure to X-ray or somewhat later. Newly formed melanophores increase rapidly in numbers to form pigmented areas often visible to the eye. By a process of degeneration, these melanophores disappear, and there results a restoration to normal coloring. At water temperatures of 70° F., depigmentation consumes roughly from two to four weeks, after which the cutaneous regions once more assume a normal color.

X-ray-produced melanophores in the goldfish behave, therefore, in much the same manner as melanophores produced in the same type of fish by trauma or in the healing of wounds or fractures as noted in earlier experiments (Smith, 1931).

It became of interest to learn what relationship, if any, melanophores newly produced from X-raying held toward groups of melanophores already existing in the goldfish in the form of cells massed to form a definite pattern of the body, head, or fins; and further, to note any evidence of degeneration in existing pattern cells following radiation.

Forty-five goldfishes, possessing various black patterns for the most part resembling those seen in Plate I, Fig. 1, were studied after exposure to 7 human erythema units of X-ray (sufficient usually to induce melanophores in the goldfish), and the behavior of melanophores in patterns and melanophores formed by X-raying were compared.

The technique of X-raying, found satisfactory in earlier experiments, was the following. The goldfish, anaesthetised in a solution

[1] Aided by Grant from Blossom Fund.

of chloretone 1 to 2000 of water, was removed from this solution and placed on a folded towel directly under the X-ray tube with the entire left side facing directly upward. One unit of human erythema dose consisted of 100 k.p.v., 5 milliampere, 8-inch target-skin distance, no filter, 72 seconds exposure. Seven erythema units involved, therefore, an exposure of 504 seconds. After exposure, the fishes were kept under conditions of ordinary laboratory light in tanks of still water (70°–78° F.) supplied with a current of air. The X-raying was done through the courtesy of Dr. William LaField, Mr. E. E. Furbush of the New Haven Hospital, and Dr. Samuel Atkins of St. Mary's Hospital, Waterbury.

The following are two illustrative experiments, in which X-ray eruptions were intense enough to permit photographing.

Experiment 1. Goldfish, 5 cm. in length from snout to base of tail (Plate I, Fig. 1, Fish *A*, photographed before exposure to X-ray) with markings of massed melanophores on head and fins. This fish received 7 human erythema units of X-ray, exposing entire left side of fish. Eleven days later an active development of melanophores occurred in the exposed surfaces of the fish. Fourteen days after exposure the X-ray eruption appeared to have reached its height and the fish was photographed (Plate I, Fig. 2). Dense masses of X-ray-induced melanophores occupied chiefly the left side of head, body, and fins, and closely encroached upon the periphery of black pigmented patterns. Depigmentation of X-ray-induced melanophores began approximately 3 weeks after exposure. Plate I, Fig. 3, shows fish 27 days after exposure with depigmentation greatly advanced. The head region has cleared, leaving the pattern undisturbed. Complete disappearance of X-ray melanophores was noted on the fifty-fifth day after exposure. Plate I, Fig. 4, was taken on the seventy-ninth day after exposure and shows head pattern practically unchanged. The pigmented markings of the fins showed microscopically no apparent difference from the original arrangement as seen before X-raying.

Although melanophores composing pre-existing patterns remain usually undisturbed by exposure to X-rays in doses of 7 human erythema units, as in the above experiment, in three instances there seemed to be definite evidence of an induced degeneration of melanophores composing a pattern, and the following illustrative experiment describes such a degeneration of pattern following a typical X-ray eruption and depigmentation.

Experiment 2.—Goldfish measuring 6 cm. from tip of snout to base of tail received 7 human erythema units of X-ray, the entire left side of the fish being exposed to the X-ray tube. Plate II, Fig. 1,

is a photograph of this fish 19 days after exposure, showing a marked massing of melanophores, *XR.*, on the left side of the head and operculum, approaching in distribution close to the small head pattern *P.* At the periphery of the head pattern, *P.*, a close intermingling occurred of both pattern cells, *P.*, and X-ray melanophores, *XR.*, seen in higher magnification in Plate II, Fig. 2. Degeneration of melanophore masses induced by X-ray began in the third week, and advanced by the twenty-seventh day to a complete clearing of the head region, the upper part of the operculum alone showing still massed X-ray-induced pigment cells, *XR.* (Plate II, Fig. 3). The head pattern, *P.*, at this time retained the details of its original form except at the caudal tip of the pattern where there was noted some degeneration of melanophores adjacent to an opaque zone (Plate II, Fig. 3, *O–O'*), a point where xanthophores had also disappeared. Plate II, Fig. 4, taken 42 days after exposure, shows the head pattern partly degenerated. Plate II, Fig. 5, 55 days after exposure, shows the head pattern no longer existing; and it was noted that the black pigmented markings on the various fins had disappeared to a very large extent. This particular fish, relatively sensitive to X-ray, showed beside melanophore degeneration, within the first two weeks a degeneration of xanthophores in several areas indicated in the photographs by the letter *O.* Zones of xanthophore degeneration appeared in life as streaky grayish opaque areas, confined chiefly to the left side of the body, which had been directly exposed to X-ray.

Ten fishes failed to give any eruption whatever of melanophores after exposure to seven human erythema units. A month later mechanical injury was produced by crushing the left operculum with an artery clamp. Five days later numerous melanophores developed near the wound in each fish, temperature of water being 76° F. It was believed that in these experimental fishes, X-ray injury was not severe enough to elicit a melanophore reaction.

EXPLANATION OF PLATE I

FIG. 1, *A*, *B*, *C*, are types of goldfishes employed in these experiments, with black pigment patterns. Fish *A*, photographed on day before X-raying.

FIG. 2, Fish *A.*, 14 days after X-raying showing pigmentation from X-ray-induced melanophores (*XR.*) chiefly on left side or exposed side, encroaching upon region of existing pattern of head, *P.*

FIG. 3, Fish *A.*, 27 days after raying. Depigmentation of X-ray-induced melanophores greatly advanced, eruption showing only at points *XR.*

FIG. 4, Fish *A.*, Photograph shows fish *A* on the seventy-ninth day with head pattern intact. Complete disappearance of X-ray eruption occurred on the fifty-fifth day.

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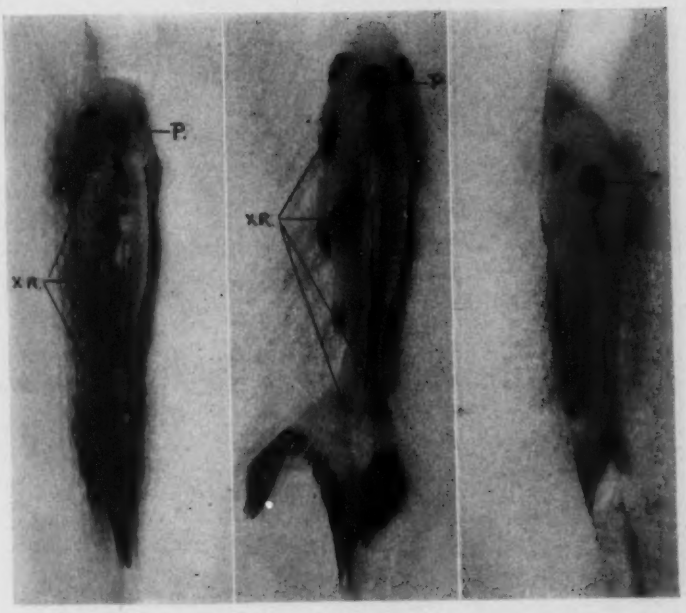

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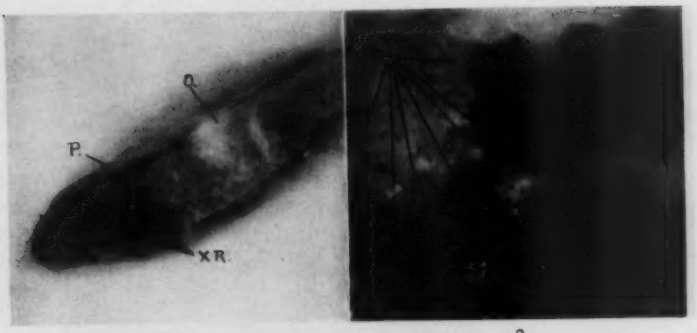
PLATE I



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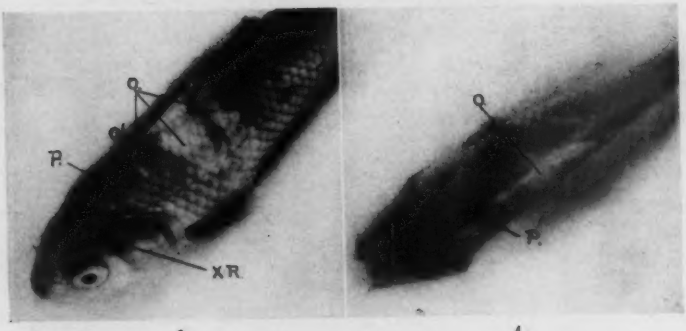

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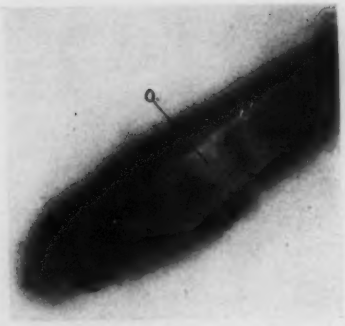

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PLATE II

Comment

Results of experiments indicated that melanophores of already existing black patterns were for the most part not influenced by single doses of X-ray as high as seven human erythema units. At the periphery of such patterns an active development of new melanophores from X-ray exposure might occur here and there, so that pattern melanophores and what may be designated X-ray melanophores grew in close apposition (Plate II, Fig. 2) with much intermingling at the time of the height of the X-ray eruption. Disappearance of the X-ray melanophore eruption left the melanophores of the pattern usually in an intact condition without alterations in the morphology of the cells. Thus, melanophores of two kinds were found to exist in the same fish, reacting differently to the effect of X-ray. On the one hand, melanophores composing existing patterns usually remained stabile, and rarely degenerated. On the other hand, masses of new melanophores evoked by X-ray, pursued a comparatively short life cycle of active growth and early degeneration with complete subsequent depigmentation.

Degeneration of pattern cells was noted definitely only three times among the 45 fishes studied, as illustrated in Experiment 2 cited above. As seen in this experiment, the head pattern slowly degenerated after disappearance of the X-ray-induced melanophore eruption. An extensive though incomplete degeneration of the fin patterns occurred simultaneously with that of the head pattern. In this particular fish, areas of degeneration of xanthophores were noted as well. It is not unlikely that further investigation will show that a dosage somewhat higher than 7 human erythema units will be neces-

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Explanation of Plate II

Fig. 1. Shows goldfish referred to in Experiment 2 with X-ray eruption (*XR.*) 19 days after raying. X-ray eruption (*XR.*) and head pattern (*P.*) are in close apposition. Letter *O* points to an area of xanthophore degeneration.

Fig. 2. A higher magnification of a part of the field in Fig. 1. *XR* points to eruption of massed melanophores induced by X-ray. *P.* represents the massed melanophores forming existing pattern of head. Melanophores of eruption and pattern intermingle at the periphery of pattern *P.* Magnification × 6.

Fig. 3. Same fish as in Fig. 1 with partial depigmentation of X-ray eruption of melanophores 27 days after X-ray exposure. The head region has cleared and shows no eruption of melanophores except at the upper part of the operculum (*XR.*) Pattern *P.* is intact except for slight degeneration posteriorly at *OO'* where xanthophores have also disappeared.

Fig. 4. Same fish 42 days after raying. Head is cleared of X-ray eruption; pattern *P.* is degenerating. Xanthophores have degenerated at point marked *O.*

Fig. 5. Same fish 55 days after raying. Head pattern has degenerated completely. Xanthophore degeneration at point marked *O.*

sary to produce uniformly degeneration of pattern melanophores of this fish, influenced by weight and size of fish.

The melanophores following X-ray exposure may bear a close morphological resemblance to the melanophores of an existing pattern, so that the two types are distinguished often with difficulty. Usually, however, X-ray melanophores look more delicate and smaller than pattern melanophores; their processes are more irregular and reach out into different planes in the tissue spaces. The pattern melanophores appear more flattened as they lie at rest spread out immediately beneath the transparent epithelium. Their borders with processes parallel to the surface appear more sharply circumscribed and deeper pigmented especially when in a somewhat contracted state.

As Fukui (1927) and Goodrich and Hanson (1931) have shown, the young goldfish is normally dark colored as the result of the presence of melanophores. Depigmentation begins irregularly after a few weeks of life and the fish gradually assumes a yellowish, golden color. The extent and completeness of depigmentation determines the pattern of adult conditions, subject probably to still further slow changes in black pigmentation later in life. Melanophores of patterns are probably fully differentiated cells, and closely affiliated with the nervous system as shown by Ballowitz (1893), von Frisch (1911) and other investigators; whereas melanophores evoked by X-ray, or by mechanical injury function perhaps in behalf of body defense and repair, when certain chemical conditions are produced in the corium of goldfishes possessing potential pigment-forming cells.

In the present experiments, areas composed of pattern cells did not seem to develop new X-ray-induced melanophores to any extent except temporarily at the periphery of the pattern. This fact suggests that conditions did not exist in the central parts of the patterns for the development of new pigmented cells, under the conditions of dosage employed, or possibly that the massed flattened pre-existing pattern cells offered enough protection against the effects of X-rays to inhibit the formation of new pigmented cells.

Summary

In the goldfish exposed to X-ray (7 human erythema units) existing patterns remained for the most part intact in the presence of an induced temporary eruption of corial melanophores caused by X-raying. In several fishes, however, a degeneration and disappearance of the patterns, partial or complete, was noted, and this followed after depigmentation of an eruption of X-ray-induced melanophores. X-ray thus produced two effects relative to melanophores, (*a*) an eruption

of new melanophores with a short life cycle, (*b*) occasional degeneration of melanophores in existing patterns.

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INTRACELLULAR CRYSTALLIZATION OF HEMOGLOBIN IN THE ERYTHROCYTES OF THE NORTHERN PIPEFISH, SYNGNATHUS FUSCUS

ALDEN B. DAWSON

(From the Zoölogical Laboratories, Harvard University, and the Marine Biological Laboratory, Woods Hole, Mass.)

It has long been recognized that the hemoglobins of different animals vary widely in solubility and ease of crystallization and that hemoglobin rarely crystallizes within the red blood corpuscles. In a previous paper (Dawson, 1930) intracellular crystallization of hemoglobin was described for the erythrocytes of the urodele, *Necturus maculosus*. In this case crystallization was apparently favored by previous poisoning with lead acetate, although it had been occasionally encountered in normal animals.

A similar phenomenon has been observed in the erythrocytes of the northern pipefish. The animals were obtained in the Eel Pond, at the Marine Biological Laboratory, Woods Hole, and appeared to be normal in all respects. The observation was made incidentally while studying supravitally the blood cells of the common marine fishes of that locality. The crystallization of hemoglobin in the erythrocytes of the pipefish is readily induced by slowly drying, in the air, rather thick smears of blood, and is most uniformly obtained when the humidity is relatively high. Preceding the appearance of definite crystals the cells lose their typical oval form and show an increasing tendency towards angularity. The majority finally assume a triangular shape but some become rhomboidal. However, at this stage the hemoglobin gives no evidence of crystal formation. Soon well-defined clefts appear in the cell contents and definite crystals then appear.

The number of crystals formed in individual cells is subject to some variation. Three crystals, forming the three sides of a triangle with the nucleus in the center, are most commonly encountered. Occasionally four crystals forming a rhomboidal figure, and more rarely two crystals arranged parallel with the long axis of the cell are present (Fig. 1). Frequently a variable number of very small, slender crystals may be associated with larger ones. They usually lie irregularly about the nucleus. The size of the larger crystals is also somewhat variable, but the shape is relatively constant. Practically all are

modified on the side next the cell membrane, being rounded rather than straight. In addition many are notched on the inner side especially if they are in contact with the surface of the nucleus.

Discussion

Little is known of the factors involved in maintaining the hemoglobin within the red blood cell in solution. In the present instance the only obvious cause of the crystallization of the hemoglobin in the

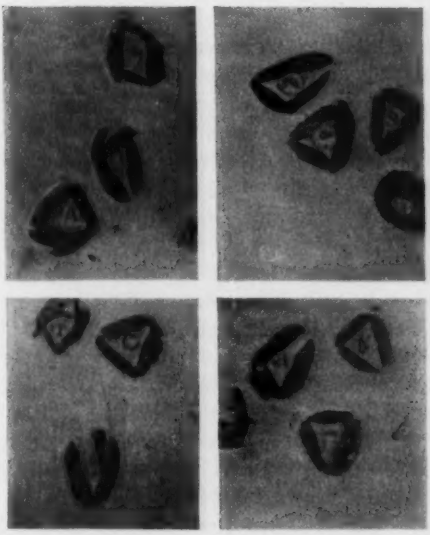

Fig. 1. Four selected areas from a preparation of pipefish blood showing the characteristic numbers, size, form, and position of hemoglobin crystals within the erythrocytes. The turbidity of the background is caused by the laking of many cells due to the injury produced in transferring the cells from the slide to a coverslip in order to obtain a preparation thin enough to photograph. Magnification × 1150.

erythrocytes of the pipefish is the slow withdrawal of water with whatever attendant injuries that may occur when drying takes place. It is of interest to note that the cell is deformed by the changed orientation of the hemoglobin molecules before any change in the nature of the hemoglobin can be observed with ordinary transmitted light and that the form acquired by the cell, triangular or rhomboidal, foreshadows the appearance of three or four major crystals within it. Moreover, the clefts which mark the amount of hemoglobin to be apportioned to each crystal also become evident while the hemoglobin still appears unmodified. One of the striking features of all erythro-

cytes is their tendency to return to their specific form after deformation, but in the case of incipient crystallization the shift in orientation of the hemoglobin molecules is sufficient to produce a permanent distortion.

In the case of *Necturus*, previously described, and in the pipefish the crystals of hemoglobin are large and relatively few. In other instances that have come under my observation while studying supravitally the erythrocytes of many vertebrates, the crystallization of hemoglobin has been quite different, the crystals being numerous and very small, producing a granular effect. Such crystallization has been encountered on a few occasions in *Necturus* as well as in another urodele, *Eurycea bislineata*. It has also been noted in several fishes such as the common mackerel, menhaden, alewife, and sea bass. In all of these cases the cause of the crystallization was unknown and appeared irregularly in preparations of fresh blood.

In a review of the literature one finds few references to intracellular crystallization of hemoglobin. Guerber (1927) observed it in the erythroblasts of embryos of the pig and cow. Kranz (1928) described crystals in mammalian erythrocytes after fixation with potassium bichromate and acetic acid, followed by paraffin imbedding. Celloidin imbedding gave negative results. He believed that the crystals were not pure hemoglobin but a product resulting from the reaction of hematin with the chromic and acetic acids. The work of Kranz was subsequently repeated by Tschachmachtschian (1932) who concluded that the crystals described by Kranz were entirely an artefact, the result of paraffin imbedding, and were not directly related to the hemoglobin content of the erythrocytes.

Jokl (1925), while studying fresh preparations of skate's blood, observed certain erythrocytes in which the cell content was divided obliquely by two or three peculiar light stripes. These light stripes appear comparable to the clefts which appear in the hemoglobin of the red cells of the pipefish, preceding the appearance of the large crystals.

Intracellular crystallization of hemoglobin was encountered in certain teleosts by Yoffey (1929), although he failed to recognize it as such. He states: "In the *Gadus* group the erythrocytes may assume a very curious shape. At first round, they then become oval, as in other fishes. They then show an increasing tendency towards angularity, and finally may become perfectly triangular in shape (Fig. 15). The relative proportion of triangular to oval red blood corpuscles varies from one animal to another. The illustration shown is from a blood film of *Gadus minutus* in which the majority of the erythrocytes

are triangular. On the other hand there are many cases in which only a few of the corpuscles are triangular, and the majority are of the normal shape. The triangularity is not artificially produced by the fixative because it may be observed in specimens of perfectly fresh and unfixed blood, though the angles may not be sharp as in the fixed film." (p. 336.)

From Yoffey's description it is obvious that in *Gadus minutus* he was dealing with intracellular crystallization and, in the photograph reproduced in his Fig. 15, three large crystals are clearly seen in almost every cell. Apparently in the *Gadus* group crystallization of hemoglobin occurs as readily as in the pipefish and was induced by Yoffey unconsciously by slight variations in his technique.

Summary

Crystallization of hemoglobin within the erythrocytes of the pipefish is described. This phenomenon is readily produced by slow drying, especially in a humid atmosphere.

Preceding the appearance of the definitive crystals the erythrocytes lose their characteristic oval form and become angular, triangular and rhomboidal forms predominating. Then definite clefts appear, followed soon after by the appearance of typical crystals. The number of crystals within individual cells varies. Two, three, and four large crystals are most commonly encountered, but a more variable number of minute needle-like forms may also be present in the erythrocyte.

It is of interest to note that the erythrocytes exhibit deformation due to the changing orientation of the hemoglobin molecules before any evidence of crystal formation can be detected with ordinary transmitted light.

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SALT REQUIREMENTS AND SPACE ORIENTATION OF THE LITTORAL ISOPOD LIGIA IN BERMUDA[1]

T. CUNLIFFE BARNES

OSBORN ZOÖLOGICAL LABORATORY, YALE UNIVERSITY

INTRODUCTION

The important problem of the invasion of terrestrial or fresh-water habitats by marine organisms has received considerable attention in recent years (cf. Schlieper, 1929; Pearse, 1929; Pantin, 1931). The Isopoda extend from great depths of the ocean to terrestrial and fresh-water environments and should afford especially interesting material for these investigations. Tait (1916) in particular has studied the longevity of the littoral form, *Ligia oceanica*, in dilutions of sea water. The structure of *Ligia* has been described in detail in the monograph of Hewitt (1907).

The present paper deals chiefly with the effect of changes in the salt content of sea water on *Ligia baudiniana* Milne-Edwards, the common isopod in Bermuda.

HABITS

Ligia baudiniana may be found in great numbers on the rocky shore (Verrill, 1903), especially in the intertidal zone at low tide. *L. oceanica* (Tait, 1916) sometimes remains covered with the tide but *L. baudiniana* retreats as the water advances. When isolated on stones in tidal pools, the isopods will run from one side of the rock to the other as if seeking a dry pathway to the shore. Occasionally I have observed them leaping from one stone to another to avoid the water. However, they are dependent on the sea water to keep the surface of the gills moist and we have never found specimens more than seventy feet from the sea; they appear in great numbers on rocks and walls several feet from the sea on rainy days. If placed in a terrarium containing a small pool of sea water, they will crawl to the edge of the water, turn around, and dip the ends of the uropodal spines in the water. By bringing the spines close together and altering the distance between the spines in a slow rhythm, the isopod moistens the gills with the water which rises between the spines by capillarity (Fig. 1). In this way a large drop of water may form on the gills.

[1] Contribution from the Bermuda Biological Station for Research.

These spines, which are usually long, are also used as swimming fins and feelers as in other species of *Ligia*.

It is probable that *Ligia* enters the sea to release the young from the brood pouch for this occurred only in submerged specimens.

An examination of the gut contents revealed vegetable debris and unicellular algae. According to Hewitt (1907) *L. oceanica* feeds largely on decaying vegetable matter. *L. exotica* is described as omnivorous by Pearse (1931). Although Pearse (1929) is undoubtedly correct in pointing out that food supply alone is probably not the most important factor in determining the habitat of littoral forms, yet it is of interest to note that *Ligia baudiniana* subsists largely on the green coating of rocks in the intertidal zone.

FIG. 1. *Ligia baudiniana* wetting the gills by the capillary action of the uropodal spines which are dipping into the sea.

CONCENTRATION EFFECTS

As a basis for comparison for subsequent experiments, the longevity of the isopods was first determined in sea water, air, and in fresh water. Individual specimens, carefully collected to avoid injury, were placed in finger bowls containing 100 cc. of water or solution. The average duration of life was only four hours in distilled water, seven and one-half hours in fresh water and thirty-four hours in sea water (Table I). The maximum longevity is also given in the tables. Sea water, changed every twenty-four hours, gave an average duration of life of fifty-eight hours, and in running aerated sea water the average longevity was one hundred and ninety-two hours, but the maximum was recorded for unchanged sea water. The large surface exposed to the air in the finger bowls permitted considerable diffusion of O_2 and CO_2 as is indicated by the maximum of twelve and one-half days in unchanged sea water. In dry glass dishes the average duration (and the maximum) was eleven hours, but in bowls containing damp sand the isopods lived for very long periods (fifteen days). It is clear that moist air is a far more favorable medium than sea water. *Ligia* dies in about one hour in dry air in the sun at 30–37° C. In sea

water the isopods survive overnight at a temperature of 5° C., but the gills cease beating at 15° C.

The life of *Ligia* in sea water is markedly curtailed by dilution below 50 per cent or by doubling the concentration of salts (Table II). In dilute sea water there is a slight increase in the frequency of gill

TABLE I

Longevity of Ligia in Air and in Water

Medium	Average Duration of Life	Maximum	No. of Specimens Tested
	hours		
Distilled water	4	5	21
Fresh water	7½	8	6
Sea water	34	297	93
Sea water (changed daily)	58	198	20
Running sea water	83	192	26
Air	11	12	8
Air over damp sand	360	625	31

TABLE II

Concentration Effects on Ligia

Medium	Average Longevity	Maximum	No. of Specimens
	hours		
25% sea water	6	10	6
50% sea water	20	70	7
75% sea water	51	172	8
200% sea water	3	7	5
250% sea water	1	1	4
400% sea water	1	2	3
M/2 glycerine	7	10	5
M glycerine	5	12	13
M/4 glycerine in sea water	11	24	8
M/2 glycerine in sea water	9	19	6
M glycerine in sea water	4	5	6
2 M glycerine in sea water	2	2	3

movements. The brief existence of the isopod in distilled water is not due to the decreased osmotic pressure, for the addition of glycerine has little beneficial effect (Table II). On the other hand, the death of *Ligia* in concentrated sea water appears to be due largely to osmotic factors as will be seen in the records of rapid death for sea water in which the osmotic pressure was increased by glycerine.

Specific Ion Effects

Of solutions containing a single salt isosmotic with Bermuda sea water (5/8 M), the isopods lived longest in NaCl, eight hours, and $CaCl_2$, seven hours; while the average duration of life in $MgCl_2$ and KCl was only four and one and one-half hours respectively (Table III). KCl exerted an immediate paralyzing effect on the gills which normally began to vibrate as soon as the animal was immersed in any of the solutions mentioned in this paper except KCl. The frequency of gill movements was taken at intervals in all solutions, but no vibrations were ever observed in KCl although the animals appeared quite normal for the first half hour. The average time for ten beats was three and one-fifth seconds in sea water (27°); and approaching death was indicated when this increased to five seconds. Immature specimens (12–15 mm.) had a faster rate, two and one-tenth seconds, and were not used; in addition it was found that immature specimens showed greater resistance to all solutions tested.

TABLE III

Specific Ion Effects on Ligia

Solution	Average Length of Life	Maximum	No. of Specimens
	hours		
5/8 M NaCl	8	14	12
5/8 M $CaCl_2$	7	13	19
5/8 M $MgCl_2$	3	5	7
2.5/8 M $MgSO_4$	4	4	10
1 M $MgSO_4$	3	4	8
5/8 M KCl	1½	3	10

Ionic Antagonism

Combinations of two ions were tried in various proportions, but no satisfactory solution was found. No specimens lived for more than a very few hours in binary mixtures but some antagonism seemed evident between Na and Ca. In solutions containing Mg or K the longevity seemed to be controlled by the amount of the most toxic ion present. In artificial sea water (*i.e.*, 100 NaCl, 11.6 $MgSO_4$, 2.2 KCl and 2.5 $CaCl_2$ in 5/8 M conc.) the duration, forty hours, compared very favorably with natural sea water. In artificial sea water containing no magnesium, the same average duration of life was exhibited (Table IV). If the KCl was omitted, the isopods lived for twenty hours; in the absence of Ca, fourteen hours; and they died within two hours in artificial sea water containing no Na.

The next step was to determine how long *Ligia* would survive if the concentration of individual ions were increased in sea water. In the case of Na, Ca and Mg concentrations not exceeding M/8 made up in sea water (*i.e.*, one-eighth of the molecular weight added to a liter of sea water) did not exert an appreciable toxic effect but KCl showed a limiting concentration of M/10 in sea water (Table V).

TABLE IV

Longevity of Ligia in Antagonistic Solutions

Solution	Average Length of Life	Maximum	No. of Specimens
	hours		
Artificial sea water	40	123	8
Same without Na	2	2	5
Same without Ca	14	23	10
Same without K	20	28	9
Same without Mg	41	120	22

TABLE V

Effect of Increasing Concentration of Ions in Sea Water

Solution	Average Longevity	Maximum	Number of Specimens
	hours		
5/8 M NaCl made in sea water	9½	36	10
1/4 M NaCl made in sea water	11	27	5
1/6 M NaCl made in sea water	6	11	8
1/8 M NaCl made in sea water	57	244	5
2.5/8 M $CaCl_2$ made in sea water	1½	2½	9
1/8 M $CaCl_2$ made in sea water	88	248	8
2.5/8 M $MgCl_2$ made in sea water	3	6	5
1/8 M $MgSO_4$ made in sea water	91	258	5
5/8 M KCl made in sea water	2	3	13
1/4 M KCl made in sea water	3½	5	9
1/6 M KCl made in sea water	5	5	9
1/8 M KCl made in sea water	6½	16	9
1/10 M KCl made in sea water	31	201	14

The gills were completely inhibited in 5/8 M KCl in sea water but exhibited the usual rhythm in M/4 KCl in sea water. In all these solutions in which salts were added to sea water it is probable that the increased osmotic pressure was significant judging from the short life of isopods in sea water containing glycerine (Table II).

SPACE ORIENTATION

Ligia baudiniana inhabits a very restricted zone along the shore line, and a number of experiments were performed to discover what

tropisms or other reactions restricted the distribution of the isopod. One may mention first the inability to survive in sea water or in dry air and the presence of food (unicellular algæ) on the intertidal rocks. These facts, however, do not explain the curious ability of the animal to orient towards the sea when released a short distance from the shore. It was noted that the isopods appeared to be reacting to the inclination of the land sloping gradually to the sea and it was found that under controlled conditions (in a photographic dark room under dim red illumination) pronounced geotropic orientation was exhibited.

TABLE VI

Orientation of Ligia on Slopes near the Sea

Date	Temp.	Direction of Slope	Inclination	Number Released	Direction of Creeping
	° C.		*degrees*		
July 10	27	Towards sea	30	6	5 down
11	27	Towards sea	40	2	down
11	27	Away from sea	40	2	down
Aug. 1	26	Towards sea	40	9	7 down
					1 up
					1 went up but turned
12	27	Away from sea	30	6	5 down
14	25	Towards sea	40	5	down
14	25	Right angles to sea	60	4	3 down
14	25	Away from sea	20	4	3 down
					1 up
14	25	Away from sea	45	4	2 straight down
					3 down obliquely
24	28.6	Away from sea	60	4	3 down
25	27	Towards sea	50	3	down
25	27	Away from sea	20	5	4 down
25	27	Away from sea	30	10	4 down
					6 up

To test this hypothesis, specimens were released at various distances from the sea on ground (sand, grass, or rocks) sloping towards and also away from the sea (Table VI). Of sixty-four isopods tested, fifty showed positive geotropic orientation and crawled downward regardless of the direction of the sea. They also showed a less pronounced tendency to crawl in the direction of open patches of sky and exhibited positive phototropism under controlled conditions.

When isopods are released in the sea at a distance not greater than eight feet from shore, they swim energetically to shore or crawl over the bottom directly to shore. In general, the animal crawls over the bottom and seldom exhibits the typical swimming movements unless

in deep water. The cause of this orientation to shore in the sea is unknown; it is independent of currents or the direction of the sun. The animal is negatively rheotropic and will swim against currents in an aquarium even after the removal of antennæ and uropodal spines, but this has nothing to do with the shoreward orientation. Luther (1930) has shown recently that the antennules of crabs are receptors for rheotropism, but in *Ligia* the antennules are extremely small (cf. Hewitt, 1907), and it seems probable that currents in the water stimulate the legs.

Discussion

Ligia affords a striking example of a marine organism which is invading the land through the intertidal zone—an approach to terrestrial life which has not received sufficient attention in theoretical considerations of the evolution of land animals. In tropical islands, lacking fresh water, and where there is no pronounced temperature difference between sea and air, the intertidal zone becomes an important route for the invasion of the land (Pearse, 1929). *Ligia baudiniana* contrasts sharply with *L. oceanica* which, according to Tait (1916), may live over eighty days in sea water. However, like the beach crab *Ocypode*, *L. baudiniana* is dependent on sea water to keep the gills moist for aerial respiration. It also resembles the beach crab in its inability to withstand fresh water or diluted sea water, and is thus quite different from the marine *Gammarus*, which lives long periods in sea water diluted to .5 per cent (Adolph, 1925). The death of *Ligia* in distilled water and in glycerine solutions indicates that, like *Gammarus* (Loeb, 1903), loss of essential salts is more detrimental than osmotic disturbances in the medium. In spite of its terrestrial life, *Ligia baudiniana* is clearly a poikilosmotic form, although in diluted sea water the respiratory rate increases, which on Schlieper's (1929) theory might be due to osmotic work performed in partially resisting disturbance of the water and salt equilibrium.

The order of toxicity of single ions, $K > Mg > Ca > Na$, appears to be about the same for several Crustacea, *i.e.*, *Gammarus* (Adolph, 1925), *Artemia* (Martin and Wilbur, 1921), *Daphnia* (Berger, 1929), *Cambarus* (Helff, 1929), and is the reverse of the toxicity series for the egg of the sea urchin (Page, 1929). The rapid death in KCl is probably due in part to the lack of ventilation of the gills, which are unable to move in this solution. Zoond (1931) has shown that ventilation of the gill surface is of vital importance in Crustacea due to the extremely slow rate of diffusion of O_2 in water. The recent experiments of Bialaszewicz (1932) have demonstrated that the high toxicity of KCl for Crustacea is associated with its rapid disappearance from the blood

nto the tissues. According to Loeb (1903) Na, K, and Ca are necessary for the gill movements of *Gammarus*, but it resembles *Ligia* in certain other salt requirements, *i.e.*, there is no satisfactory binary mixture and Mg appears to be a dispensable ion.

The orientation of *Ligia* to the sea resembles that of young loggerhead turtles described by Parker (1922). The orientation of the isopod to the shore, when in the sea, appears to be an instance of definite orientation which is not governed by a simple type of tropistic response and is not unlike the orientation of *Onchidium* (Arey and Crozier, 1921), and of ants (Barnes, 1929). The fact that the orientation of *Ligia* in the sea is not exhibited at distances greater than ten feet from the shore precludes the possibility that the isopod reacts to the blue color of deeper water as in the loggerhead turtle (Hooker, 1910).

Summary

1. *Ligia baudiniana* moistens its gills by the capillary action of the uropodal spines.

2. The isopod survives best in damp air and is unable to live for long periods in sea water.

3. Changes in the concentration of sea water are detrimental, *i.e.*, *Ligia* is poikilosmotic.

4. The cations of sea water are toxic in the following order: K > Mg > Ca > Na. K exerts a specific paralyzing effect on the gill movements.

5. On land *Ligia* orients toward the sea. Positive geotropism appears to be the most important factor.

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THE RELATION BETWEEN ABSORPTION AND ELIMINATION OF WATER BY TERMOPSIS ANGUSTICOLLIS

S. F. COOK AND K. G. SCOTT

(From the Division of Physiology, University of California Medical School, Berkeley, California)

The purpose of this investigation was to study the water relations of the termite from the point of view of the animal itself. The question has been of interest in its ecological aspects with reference to the climatic conditions favorable for termite growth and to moisture and dryness as factors determining the distribution of species. As far as we are aware no studies have been undertaken to determine under controlled conditions from what source the termite derives its water, how it undergoes water loss, and how much drying it can suffer without ill effect. These questions are considered in the data here presented.

The organism used was *Termopsis angusticollis*, the common large wood termite of the Pacific Coast. The termites were collected in the vicinity of Berkeley and kept in jars of moist rotten wood until needed. The first step was to determine the normal water content of *Termopsis* and establish the relation between the partial pressure of the water vapor in the atmosphere surrounding the termites and their water content.

Experiment 1. Three groups of 50 termites each were placed in desiccators. In the desiccator with Group I was placed a vial containing water. At equilibrium, therefore, the relative humidity was 100 per cent and the partial pressure of water vapor from 15 to 17 mm. Hg, since the temperature varied from 17° to 20° C. It was not possible to maintain an absolutely constant temperature, but the variation of approximately 3° was too slight to affect the validity of the result.

In the second group the vapor pressure was lowered to 7 to 9 mm. Hg, the variation again being dependent on the temperature. This was accomplished by placing in the desiccator with the termites a saturated solution of calcium chloride over solid calcium chloride. The vapor pressure should theoretically be of the order of magnitude mentioned. This was checked by evacuating a vessel containing a similar mixture of solid and dissolved calcium chloride and allowing the system to come to equilibrium, the actual pressure of the water vapor being read on a mercury manometer. The use of this mixture

is also of advantage since the absorption of water from the termites does not alter the concentration of the dissolved calcium chloride when the solid phase is present in excess. Since the termites were transferred directly from conditions of water saturation to this tension, any effects due to the reduced tension should become immediately apparent.

The third group of termites was placed in a desiccator with anhydrous calcium chloride where the vapor tension was less than one millimeter.

All three groups of termites were kept under the conditions observed above for two days. Food was provided in the form of punky wood which had been allowed in each case to come into equilibrium with the appropriate vapor tension before the experiment began. Starvation, therefore, was eliminated as a factor in reducing the weight

TABLE I

Water loss by termites at different vapor tensions. In each case the initial number was fifty.

A.	Group I	Group II	Group III
B. Vapor tension in mm. Hg	15–17	7–9	0–1
C. Average fresh weight in mg.	42.4	29.25	34.5
D. Average weight after experiment in mg.	39.8	24.8	26.3
E. Average dry weight after experiment in mg.	11.1	5.9	8.0
F. Water loss in per cent: $\frac{C-D}{D} \times 100$	6.2	15.3	23.1
G. Water content of termites at beginning in per cent: $\frac{C-E}{C} \times 100$	73.8	79.9	76.8
H. Water content of termites at end of experiment: $\frac{D-E}{C} \times 100$	72.2	76.1	69.6

of the termites. The termites were weighed before and after the exposure to the different degrees of humidity and the results expressed as average weight per termite. This was permissible because there was a sufficiently large number of animals in each case (50) to justify an average. Finally the dry weight was determined. The data are summarized in Table I.

From this table it is evident that the percentage of water lost is inversely proportional to the water vapor tension. Furthermore, the water content and the average weight appear to stand in inverse ratio. This probably can be explained by the fact that the older insects have developed wing pads and a thicker integument, thus lowering the relative water content. Nevertheless the extreme variation in relative water content which may be ascribed to this factor is of the order of 6

per cent (Line *G*, Table I), whereas the extreme observed variation is approximately 17 per cent (Line *F*, Table I). There is little doubt, therefore, that the water loss is substantially proportional to the decrease in vapor tension. It is highly probable that most of this water is lost through evaporation from the tracheæ and the body surface.

Experiment 2. In order to check the above results, an experiment was performed in which the water actually given off by the termites was compared with the loss in weight of the animals. Fifty termites were placed in a test tube through which dry air was passed for 212 hours. Before reaching the animals the air was dried thoroughly by passing it through ten feet of one-inch glass tubing filled with anhydrous calcium chloride and then through a flask of pure, concentrated sulphuric acid. The water and carbon dioxide given off by the termites was absorbed by anhydrous calcium chloride of the finest mesh obtainable, packed tightly in a medium-sized U-tube. Since the total quantity of carbon dioxide and water produced was small, and since the rate of flow was very slow, there can be little doubt that the absorption by the calcium chloride was practically complete. The carbon dioxide and water were not determined separately because the average carbon dioxide production of *Termopsis* has already been determined by one of us (Cook, 1932). According to the data presented in the paper mentioned, the average production is approximately 8.9 milligrams per gram termite per hour. The calculated value of the carbon dioxide produced during any time interval could then be deducted from the gain in weight of the calcium chloride with the reasonable assurance that the balance represented water. The initial weight and that at the end of the experiment were obtained, also the weight of food consumed (filter paper) and the weight of the feces (see Table II). Under the conditions described, viz., a very slow current of air, the water loss proceeded at a fairly regular but diminishing rate until the effects of the drying were apparent in the behavior of the termites. The effect of rapid drying is described elsewhere.

The total gain in weight of the calcium chloride in 212 hours was 628 milligrams, of which 150 milligrams (0.369 milligram per hour x 212 hours x 1,910 milligrams) may be ascribed to carbon dioxide, leaving 478 milligrams as the weight of the water. Meanwhile the loss in weight of the termites was 369 milligrams.

The excess water found may be accounted for in two ways. In the first place, since the diet was almost exclusively cellulose, and had been for weeks previous, and since the R.Q. under such conditions is very nearly unity (Cook, 1932), the predominant oxidation must have been that of carbohydrate. This should, of course, yield one molecule of

water to each molecule of carbon dioxide, and if the total weight of carbon dioxide was 150 milligrams, as suggested above, the corresponding weight of the water produced would be 61 milligrams. In the second place, the three termites which died and were eaten must have contained, on the basis of the data presented with Experiment 1, about 75 per cent of water, or 90 milligrams. This water must have appeared in the calcium chloride tube directly by evaporation from the three termites while living or dead, or have been consumed by the others. In the latter case it would have been lost again from the surviving animals before the completion of the experiment. These two sources of water combined would furnish, therefore, a maximum of 150 milligrams, which when subtracted from the total of 478 milligrams found equals 328 milligrams. This is comparable with the net loss in weight of 369 milligrams.

TABLE II

Water Loss by Fifty Termites in a Slow Current of Dry Air

Initial total weight	Final weight	Filter paper consumed	Feces	Water lost in indicated intervals	Total water lost at end of indicated periods
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
2,030	1,541	105	92	83 in 19 hrs.	83 in 19 hrs.
				83 in 23 hrs.	165 in 44 hrs.
				43 in 14 hrs.	208 in 58 hrs.
				29 in 10 hrs.	236 in 68 hrs.
				47 in 24 hrs.	283 in 92 hrs.
				78 in 48 hrs.	360 in 140 hrs.
				77 in 48 hrs.	438 in 188 hrs.
				40 in 24 hrs.	478 in 212 hrs.

The water loss obtained in Group III of Experiment 1 was 23.1 per cent. In this experiment the loss as based on the water found in calcium chloride is 19.3 per cent, and as based on the loss in weight is 17.2 per cent. The results obtained by the two methods of estimation in Experiment 2 show a reasonably close correspondence. The difference between the degree of water loss in Experiment 1 (23.1 per cent) and in Experiment 2 (19.3 and 17.2 per cent) is not sufficient to invalidate the conclusion that under prolonged conditions of dryness the termites lose water to the extent of approximately 20 per cent of their weight.

Experiment 3. The water loss having been shown to be primarily through evaporation, the mode of intake was next investigated. There are but two possibilities. The water must be absorbed from the atmosphere or taken in with the food. If the termite can use

atmospheric water, it must be able to absorb the vapor from a fully saturated atmosphere. If it can supply its needs adequately in this way, it should be able to utilize very dry food. If it cannot, then at least some water must be presented in the liquid form with the diet. To differentiate between these two possible sources of water, three groups of 25 termites each were kept under different conditions of atmospheric and food moisture. All three groups were fed wood which had been dried in a desiccator over calcium chloride for several days prior to the experiment. Owing to the great power of cellulose to absorb water even from the most powerful drying agents known, the water content could not have been zero, but it was lowered sufficiently to demonstrate the inability of the termite to live on relatively dry food.

It is obviously impossible to feed termites dry wood in a saturated atmosphere or damp wood in a completely dry atmosphere. Therefore the separation of food and atmospheric water had to be made in *time*. This was done as follows: Group I was kept 22 hours per day in saturated air without food, and was placed in a desiccator with dry air and dry food two hours. Group II was exposed to moist air 16 hours and to dry air and food 8 hours. Group III was exposed to each set of conditions 12 hours per day. In no case could the termites get much water from the food, but if it is possible for them to utilize dry food and atmospheric water, then at least Group I should be able to absorb as much water in 22 hours at a high humidity as it would lose during two hours at low humidity, and thus the normal water content should be maintained. But if they are wholly dependent on food moisture for their water intake, then all the groups might be expected to lose water irrecoverably during the period of exposure to dryness.

The water loss was determined by weighing after four days, but the termites were kept under the same conditions until they died in order to secure information concerning their viability. The data are presented in Table III. It will be observed that the water loss in all groups is substantially the same and is of the order of magnitude observed in the two previous experiments (20 to 23 per cent).[1] This seems to be the case even though the periods of feeding varied so that in Group I the termites had only two hours to lose water and twenty-two in which to regain it. But they lost as much water in four feedings, or a total of eight hours, as Group III, which was placed in dry air six times as long.

[1] The effect of starvation in four days may be neglected, particularly in view of the fact that in Experiment 2 it was shown to play no significant rôle even in eight days.

The substantially equal loss of water with such widely varying total time of exposure to dryness as 8 and 48 hours presents an apparent anomaly. But this anomaly in itself shows that the water loss in dry air and dry food is *cumulative* and that intervening periods in damp air of as long as 22 hours per day do not tend to decrease the entire water loss incurred during the combined time of the dry feeding periods, even though this total time is as short as 8 hours. In other words, after losing water during a short exposure to dryness the termites cannot make up the loss by even a long sojourn in a highly saturated atmosphere. This view is further substantiated by the data presented in Experiment 4, below, from which it is evident that rapid drying extracts the major portion of the water in about eight hours. In Group III the desiccation of the animals probably reached

TABLE III

The Water Loss and Viability of Termites under Varying Degrees of Atmospheric and Food Moisture

	Group I Moist air 22 hours per day. Dry air and food 2 hours	Group II Moist air 16 hours per day. Dry air and food 8 hours	Group III Moist air 12 hours per day. Dry air and food 12 hours
Average initial weight in mg.	33.4	37.3	33.7
Average weight after 4 days in mg.	26.0	29.6	25.9
Water loss in per cent	22.4	20.8	23.1
Per cent mortality after days specified: 4	8	4	0
6	48	4	4
8	80	44	24
12	96	96	76
15	100	100	92

at the end of the first 8 to 10 hours the same degree as that attained by Group I at the end of four days. But with Group III the remaining forty odd hours of dryness did not serve to make any material additions to the water loss. Experiment 4 indicates that at this stage of drying water becomes very difficult to remove and it also must be remembered that the animals of Group III were consuming considerable amounts of cellulose which, although dry, nevertheless must have contained a slight quantity of hygroscopic water.

From these findings the conclusion seems to be justified that the termite cannot utilize the water in the atmosphere to *replace* that lost by evaporation. The viability data indicate that during prolonged exposures death occurs primarily as a result of drying, and secondarily, from the starvation which accompanies it. The course of mortality

was similar in all three groups but life was most prolonged in the twelve-hour group, and least prolonged in the two-hour group. Since the former had a longer feeding period, it seems possible that they received slightly more nourishment despite the general unavailability of the dried wood. Nevertheless, the fact that practically none survived more than fifteen days is additional evidence that dry food, even when accompanied by long periods of atmospheric saturation, will not support these animals, and that the moisture in the atmosphere can function only to prevent evaporation and thus to *diminish the water loss*. If now we exclude the water vapor surrounding the termite as a source of water we are forced to conclude that normally the water is taken in with the food.[2]

Experiment 4. The uniformity in the water loss of the termites in Experiment 3, even with a 600 per cent variation in time of exposure to very dry air, raises the question of just how rapidly the loss of water occurs, for apparently the first 20 per cent of the water is lost within 8

TABLE IV

Rate of Water Loss in Termites under Dry Conditions

Hours in desiccator	Average weight per termite	Water loss	Rate of water loss
	mg.	*per cent*	*mg. per hour per termite*
0	51.0	0	
4	45.3	11.2	1.4
8	39.9	21.6	1.3
18	36.7	28.4 (Eight dead)	0.35

hours. To make certain of this point 25 termites were placed in a desiccator over anhydrous calcium chloride and were weighed at frequent intervals. No food was given. From Table IV it may be seen that the total water loss rose as high as 28 per cent after 18 hours exposure, but that the termites were rapidly dying. Since there was no food, it was impossible for the termite to get water, even in traces from wood, but the sharp fall in water content to a 20 per cent loss in 8 hours fits in with the result obtained in the previous experiment. In fact, here the death rate was even more rapid than in Experiments

[2] Concerning the form in which the water may be ingested it may be pointed out that in addition to "free" water in the intercellular spaces of wood the hygroscopic water may be of importance. In short leaf pine at a relative humidity of 100 per cent (but with no condensation) the amount of water held in this way is approximately 30 per cent (Schorger, 1926). All forms of cellulose may absorb hygroscopic water up to a concentration of 17 per cent. The ability of the termite to exist in relatively dry places (but not completely dry) may depend upon the availability of this water after digestion of the cellulose in the gut of the termite.

2 and 3 (18 to 20 hours compared with 10 to 15 days), a difference which again may be correlated with the presence or absence of small amounts of water contained in wood. Our observations indicate that the termite survives a water shortage quite well until the loss reaches about 20 per cent of the normal fresh weight. At this point the animals begin to become moribund. Death is certain when the loss reaches about 28 per cent.

In conclusion, the chief results of this study may be briefly summarized. *Termopsis* loses water rapidly when placed in dry air, and unless the loss is compensated it may be fatal. The water for replacement is provided as liquid in the food. The animal is unable to take up water vapor actively from even a moisture-saturated atmosphere.

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